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DEVELOPMENTAL STUDIES OF THE APPLE FRUIT IN THE VARIETIES McINTOSH RED AND WAGENER

II. AN ANALYSIS OF DEVELOPMENT[1]

BY MARY MACARTHUR[2] AND R. H. WETMORE[3]

Abstract

Growth in the various tissues of the fruit of a McIntosh Red and a Wagener tree, both self-pollinated, is compared. For several days succeeding pollination no increase in fruit size is apparent. Fertilization is followed by general cell division and cell enlargement. The period of cell division varies with the tissue and with the variety. Final cell size is reached first by the cells of those tissues near the centre of the apple. Impressed upon the fundamental pattern of growth is the localized activity of the primary vascular bundles, the cambia of which add cells to the ground tissue. Angulation in the Wagener is accentuated by this activity. With the exception of cells of the epidermis, final cell size is approximately equal in comparable regions of the two varieties. Differences in regional extent are due to differences in numbers of cells in that region.

Specific size and form of an organ are achieved by the interplay of those integrating and co-ordinating processes which control development. Since such processes are dynamic, successive stages of development should form the basis of an analysis of growth in that organ. An initial stage in the study of the attainment of specific size and form in the fruit of the apple varieties, McIntosh Red and Wagener, was presented in an investigation (4) of the development of the vascular anatomy in these varieties. A logical sequence to this study is the investigation of the growth of the fruit as a whole and of the various tissues comprising that fruit. With this end in view, there were assembled measuremental data on the direction and extent of localized growth (involving cell division and cell enlargement), the differentiation and organization of the cells into tissues, and the relative importance of these tissues in the production of the mature fruit. The consideration and the interpretation of these data are reported in this paper.

As far as the authors are aware, previous investigations of the relation between cell size and organ size in apples have been made on the mature fruit alone. In the variety Bramley's Seedling, Smith (6) found a centrifugal "gradient" of differences in the sizes of the cells of the various regions from pith to cortex. In a later paper (7) he attributed final fruit size to *(a)* the amount of cell multiplication, and *(b)* the degree of cell enlargement, wherein

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either factor might be the dominant one. Houghtaling (2) in a study of the developmental anatomy of tomatoes, showed that growth in the early stages is due chiefly to an increase in cell number, later growth to an increase in cell size, and that both processes operate more extensively to produce the larger-sized fruits. In an analysis of the genetical basis of size inheritance in the tomato, MacArthur and Butler (3) state that the size differences of different species or varieties are due to rate genes that control cell number and cell size. Differences in ovary size at anthesis are due to differences in cell number, whereas differences in the size of the more mature fruit are due also to the fact that maximum cell expansion varies greatly in the several varieties during postanthesis. Sinnott's investigations on the morphogenesis of *Cucurbita Pepo* (5) take into consideration the various tissues comprising the fruit. In 12 races of squash, larger fruit size at maturity was due to a greater duration of cell division and to a greater amount of cell expansion. Various tissues increased at different rates.

Materials and Methods

At the Experimental Station, Kentville, N.S., collections of developing fruits were made throughout the growing seasons of 1935 and 1936 from a selfed McIntosh Red and a selfed Wagener tree pollinated in 1935 on June 8 and in 1936 on May 26. Fixation and staining methods were those used by

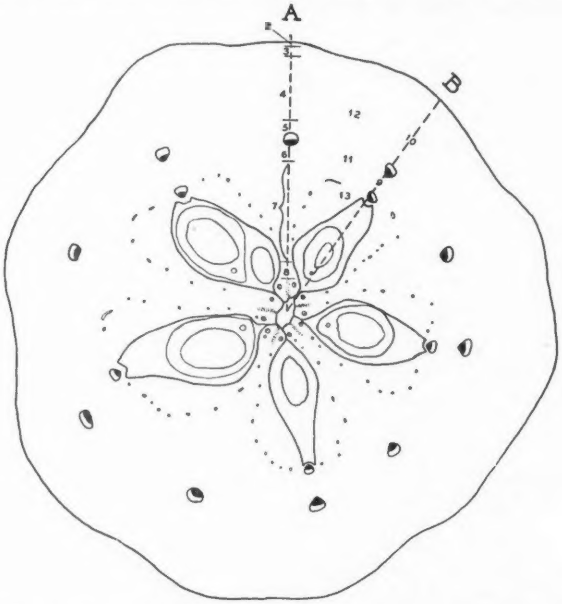

FIG. 1. *Diagram of median transverse section of the apple showing petal and sepal radii A and B, and the location of the Regions 1 to 13 used in the measurements.*

MacArthur and Wetmore (4). Only the median transverse sections were used in the measurements. An Edinger drawing apparatus was used to project the sections on paper. Cell size of tissues along and between the petal and sepal bundle radii (*A* and *B*, Fig. 1) were measured. Calibrated rules had been prepared for each combination of ocular and objective. Owing to the fact that there was considerable variability in the diameters of the cells in any one tissue, due principally to the differences of levels at which the cells were cut, the very small cells were avoided. Sample lots of 10 cells were selected in each region. Each of these cells was measured on its longest diameter, and again at right angles to this, and the two dimensions were averaged. Mean diameters of the cells of each region studied, radial widths of those regions, and the radial dimension of each entire section were the measurements used in the computations.

Observations

TISSUE CHARACTERS

Region 1

At no time in the stages examined do the epidermal cells (Region 1) of the McIntosh reach the size of those of the Wagener. The radial diameter of the epidermal cells of the McIntosh remains approximately the same from one week previous to anthesis until six days after pollination when an increase is then initiated. Three days after pollination the epidermal cells of the Wagener initiate their slow, steady increase in radial diameter. The tangential diameters in both varieties are equal and remain about the same until the end of June. From June 24 on, this latter diameter in both varieties increases and finally surpasses the radial diameter. The present observations confirm those of Tetley (8), that throughout the period of growth the layer of cutin limiting the external face of the epidermal cells gradually thickens.

Region 2

The subepidermal layer (Region 2) is at first one or two cells in thickness. In the very early stages there is no definite orientation of the longest diameter of these cells. At the time of increase in the radial thickness of the epidermis (see above), the subepidermal cells are oriented so that their longest diameter is perpendicular to the radius of the apple. Shortly after anthesis, the radial extent of this tissue increases slightly, first by periclinal cell division, then by cell enlargement. Cell enlargement is mainly effected by the tangential elongation of the cells since the radial diameters of these cells remain practically the same. The cell walls become very thick and no intercellular spaces are visible until early in August.

Region 3

In the outer cortex (Region 3) the cells are more or less isodiametric at the time of pollination. The outer boundary is recognizable early* by the large intercellular spaces similar to those reported by Bonne (1) for *Pyrus*

** The earliest buds studied were collected April 29. Already, the intercellular system was apparent in this region.*

communis and termed "la zone corticale lacuneuse." In the bud and young fruit this layer with its extended intercellular areas resembles the mesophyll tissue of a leaf. As the cells divide and enlarge this distinction is lost. The outer cortex is interpenetrated by numerous small vascular bundles each of which is surrounded by a sheath of parenchyma. The cells of this tissue merge into those of the cortex.

Region 4

The cortex proper (Region 4) is traversed by the larger branches of the anastomosing vascular system. Early in ontogeny the cells are nearly isodiametric. Later, they become roughly elliptical in shape and a radial orientation is visible. The arrangement of the cells in this tissue and the orientation of the cells themselves are probably due to the centrifugal direction of growth. This region is one showing great increase in absolute extent during the growth of the fruit.

Region 5

In the bud there is no definite orientation of this tissue which limits the outer face of the petal bundle. The cambium of the bundle, which is active in the later stages of growth, cuts off parenchyma cells externally. With this activity radial orientation is initiated.

Region 6

This is the region that limits the inner face of the petal bundle. The activity of the cambium of the bundle in cutting off parenchyma cells internally makes this region the mirror image of Region 5.

Region 7

The cells of the ventral lobe of the carpel region are radially oriented from the time of inception of the ventral lobe (4). In the later stages of growth, the staining reaction of this region differs from that of the outer tissues; starch grains are not as numerous as in Region 4, and, after the cells have reached their maximum enlargement, further increase in the radial dimension of the tissue is due to the enlargement of the intercellular spaces. This increase causes distortion of the radial arrangement of the cells.

Region 8

This tissue is made up of small cells, diversely oriented. Its distance from the centre of the apple varies according to the length of the indentations of the stylar canal. These indentations in Wagener are indicated either by an opening into the ventral lobe or by tangentially oriented, interlaced cells which in the later stages of development generally change to stone cells. In the McIntosh, because of: *(a)* the irregular orientation of the ovules (4), and *(b)* the frequent occurrence of one concentric ventral bundle serving two adjacent locules and occupying a median position in the ventral lobe, the indentations of the stylar canal in the carpel region are often absent.

Region 9

The tissue of Region 9, on Radius *B*, lies between the dorsal carpel bundle and the sepal bundle. The cells are oriented in a manner similar to that in Region 6. Its increase in extent is due to the activity of the cambium of these two bundles in cutting off parenchyma cells.

Region 10

The cells of the cortical region on the sepal bundle radius are in all respects similar to those of Region 4 on the petal radius.

Region 11

This region differs in the two varieties. It presents an angular outline, extending into the cortical tissue between sepal and petal bundle in the McIntosh. In the Wagener it is roundly lobed. The cells have no definite orientation in the early stages, but in the later stages show a certain tangential orientation, apparently brought about by the increase of pressure during the active growth of the cortex externally, and the ventral carpel lobes internally. The cells of Region 11 stain heavily.

Region 12

The tissue of Region 12 is similar to and continuous with that of Regions 4 and 10.

Region 13

This is the fleshy region of the carpels, extending from the endocarp to the carpellary network. It has a greater extent in the Wagener than in the McIntosh. Those cells that lie close to the endocarp are at first cubical. During enlargement they become several times longer than wide and the longer diameter is roughly parallel to the cartilaginous endocarp. The outer cells of the tissue divide periclinally and are finally indistinguishable from the cells of Region 7.

Interpretation of Results

A graphical summary of enlargement along Radius *A* is given in Fig. 2, where radial increase is plotted against time. At the time of anthesis the Wagener fruit is slightly larger than that of the McIntosh. In the former, on the third day after pollination, a slow increase in size begins. For the succeeding three days the increase is gradual, but with the drop of the petals the increase becomes rapid and continues at approximately this rate until towards the completion of growth.

On the other hand, the McIntosh shows no corresponding increase in size in the early stage after pollination, but in the succeeding stage a similar abrupt change is suggested though the material was not sufficient to demonstrate this point. The delayed growth response in the McIntosh may be associated with its known high self-incompatibility.

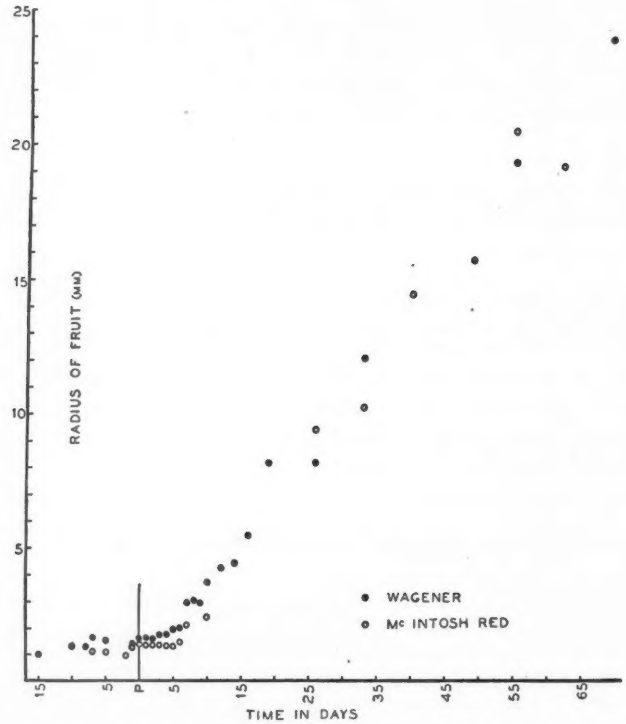

FIG. 2. *Increase of radial extent of the fruits of McIntosh Red and Wagener with respect to time. P = pollination.*

Six days after pollination the unfertilized ovules begin disintegration. Later collections of fruits with no fertilized ovules show progressive stages of fruit disintegration, this disintegration beginning in the outer cortex. These are the fruits that constitute the first "drop". In contrast, in those fruits with fertilized ovules the sudden increase of growth is apparent after the slow period. The rate of growth with respect to time is approximately the same in the two varieties. The tissues that show the greatest increase in size in the early stages are Regions 4 and 7. Later, the cambium of the petal bundle adds to the ground tissue by increasing Regions 5 and 6. Since the line of division between the cortex and the carpel region follows the inner limits of Region 6, Regions 1 to 6 inclusive have been grouped additively for the purpose of comparison with Region 7. The total width of: *(a)* these outer tissues (Regions 1 to 6 inclusive), and *(b)* Region 7 are independently plotted against time for the McIntosh (Fig. 3) and for the Wagener (Fig. 4). No attempt has been made to fit a curve to the points. Variation from a smooth curve of growth is to be expected since *(a)* these are the results of

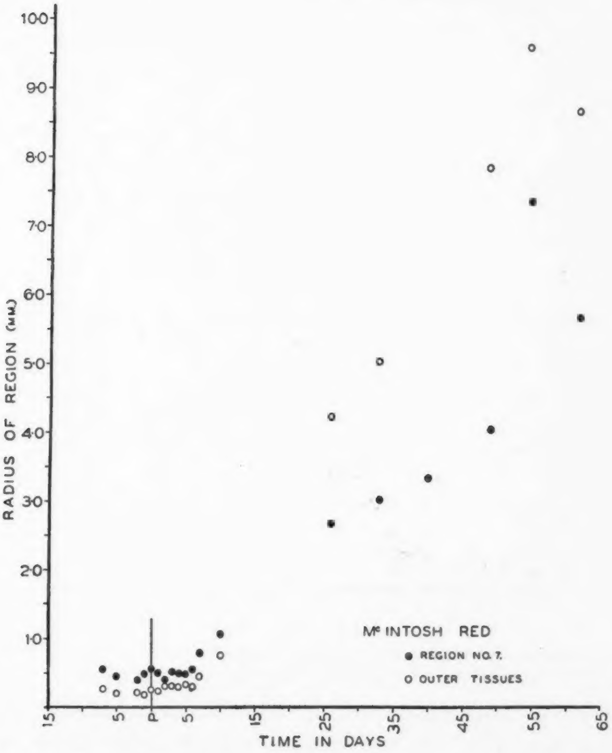

FIG. 3. *Increase of radial extent of Region 7 and of the outer tissues (Regions 1 to 6 inclusive) with respect to time in the fruits of McIntosh Red. P = pollination.*

random sampling of cells for measurement, and *(b)* the individual apples vary in size on the same date. However, the trends are indicated. Region 7, which is initiated by centripetal growth of the ventral carpel lobes (4), has a slightly greater radial extent at the time of anthesis. The slow period of growth which continues for several days after pollination is observable in both outer tissues and carpel regions. It is not as clear as in the composite graph (Fig. 2). In the McIntosh the radial extent of the outer tissues surpasses that of Region 7 at an early date. This is probably due to *(a)* the low set of seeds in the self-pollinated McIntosh, and *(b)* the "open core" condition. Direct observation reveals that the ventral bundles of those carpels with abortive ovules do not increase appreciably in diameter, whereas the bundles of those with developing ovules do, a feature that is very obvious when both types appear in the same fruit. An associated effect is the limiting of the extent of growth in the tissues external to unfertilized carpels and the increasing of it in fertilized ones, with resultant eccentricitv of shape. Moreover, the

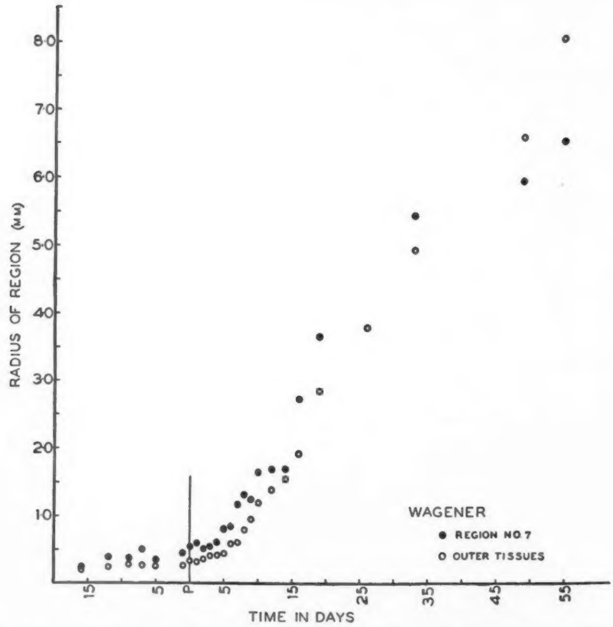

FIG. 4. *Increase of radial extent of Region 7 and of the outer tissues (Regions 1 to 6 inclusive) with respect to time in the fruits of Wagener. P = pollination.*

degree of eccentricity depends on the stage of development in the ovules when abortion occurs. For these reasons postpollination measurements are recorded only where ovules show signs of fertilization. The two regions in Wagener maintain a nearly constant rate of growth until the second week in July, or about five weeks after pollination. The scarcity of points on the graph beyond this date precludes definite conclusions, but comparison of this figure with that for the McIntosh (Fig. 3) indicates that growth of the outer tissues then surpasses that of Region 7. Moreover, this is coincident with that period in developmental history when activity in Regions 5 and 6 is apparent.

Each of the outer tissues, Regions 1 to 6, has a different mean cell dimension. Of these, the tissue having both the largest mean cell dimension and the greatest radial extent is in Region 4. In Fig. 5 the respective mean cell dimensions of Region 4 (cortex proper) and of Region 7 are plotted against the individual radial dimensions of that region. The lower part of the curve for Region 4 in the McIntosh shows a gradual increase in the mean size of the cell with an increase in the radius of the region. This is during the slow period of growth preceding and immediately following pollination, and apparently occurs before the effect of fertilization is impressed upon the tissue. The lower flattened portion of the curves for this region in the two varieties

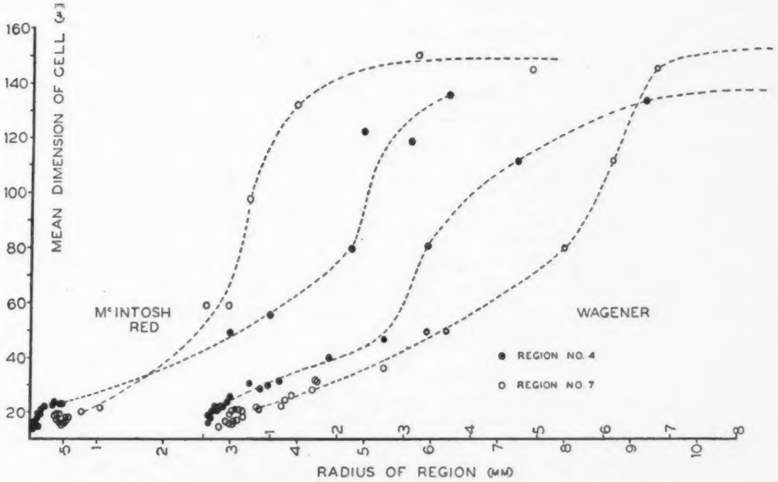

FIG. 5. *Graphs of mean dimensions of cells in Regions 4 and 7, respectively, against radial extent of the regions in McIntosh Red and Wagener.*

must indicate a period of cell division, since the radius of the region increases at a much greater rate than the mean size of the cell. In the McIntosh Red the period of cell division in Region 4 is more prolonged than in the Wagener, and is comparable to that in Region 7 in the latter. The more perpendicular segment of the curve is obviously the period of cell enlargement. Cell enlargement neither begins nor ends abruptly in either variety. Examination of the material discloses that before the steep segment of the curve, some of the nuclei are central, others are parietal. Scattered throughout the tissue are pairs of cells that are obviously the results of recent cell division. The upper portion of these curves, as cell size is again becoming a constant, represents a slight increase in mean cell dimension, and at the same time a slight increase in radial extent. Continued examination of sections indicates that this is not due to increase of cell number, but to the expansion of the intercellular spaces. In Region 4 of both varieties this dilation of intercellular spaces begins about nine weeks after pollination. Chemical tests for changes in the nature of the cell walls and more especially the cell contents might give some indication of the causes underlying the increase in intercellular volume. It is obvious that such tests should be made in the field where plenty of material at the proper stages is available.

The lower parts of the curves for both varieties indicate cell division. Little increase in cell size occurs at this time. The period of cell division for Region 7 in the McIntosh Red is similar to that for Region 4 in the Wagener. The radial extent of Region 7 in the Wagener is greater than that in the McIntosh before the rate of cell division diminishes. Cell enlargement follows, and succeeding that, the enlargement of the intercellular spaces, as in

Region 4. Cell size in Region 7 of the McIntosh very early exceeds cell size in Region 4. This does not occur in the Wagener until cell size in Region 4 has almost reached its maximum enlargement. Since there is no difference in the final cell size of comparable regions in the two varieties, and since the period of cell division in Region 7 of the Wagener is greater than that of the McIntosh, this region must contain a larger number of cells.

Fig. 6 is similar to Figs. 3 and 4. The outer tissues (Regions 1 to 6 inclusive) and Region 7, respectively, are plotted on a double log grid against the radii of the fruits. As this figure indicates, in both varieties the rate of increase of radial extent of the outer tissues is slightly higher than the rate of increase for the radius as a whole, and is also constant for the two varieties. Region 7 in the McIntosh has the same rate of increase of radial extent as the rate for the radius as a whole. On the other hand the same region in the Wagener has a higher rate than the radius as a whole for a time. This higher rate is equal to that of the outer tissues. When the regions are increasing by the enlargement of intercellular spaces, rate in Region 7 becomes lower than that for the radius as a whole.

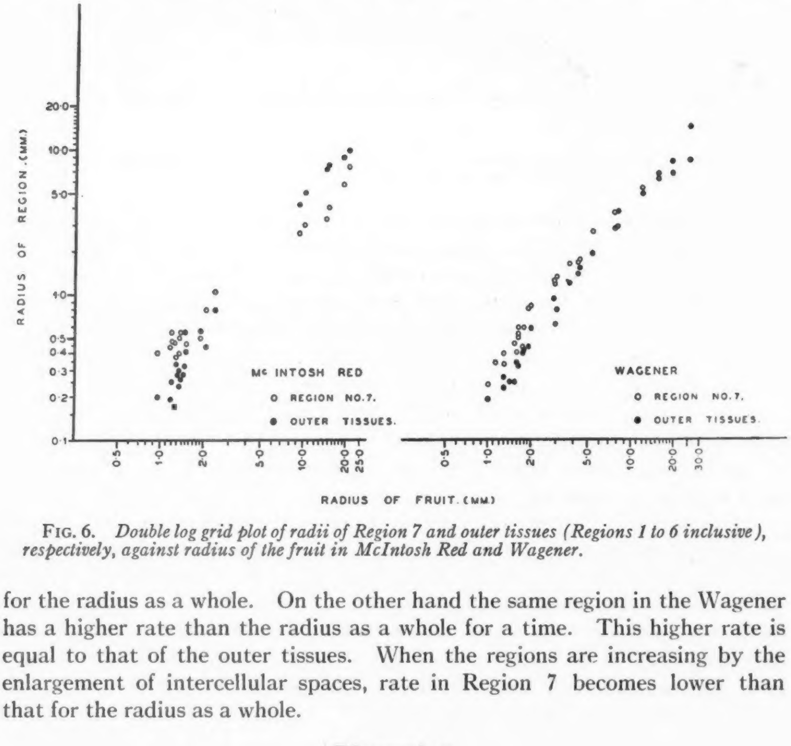

FIG. 6. *Double log grid plot of radii of Region 7 and outer tissues (Regions 1 to 6 inclusive), respectively, against radius of the fruit in McIntosh Red and Wagener.*

Discussion

An evaluation of the data just presented on the trends in the development of the apple fruit of the varieties studied demands a discussion of the effective roles of the following: (1) cell division and cell enlargement, (2) the direction and extent of localized and general growth, (3) differentiation and organization of the cells into tissues, and (4) the relative importance of these tissues in the production of the mature fruit. It is obvious that the mature fruit is not the result of the consecutive sequence of the above activities but of their integration.

Before anthesis there is a rapid development of the ovules, while the remaining tissues of the fruits increase but slightly in extent. After pollination there is a short period in which there is no significant change in the size of the fruit as a whole. This is more clearly observable in the McIntosh (Fig. 2), and may be due to the low degree of self-compatibility of the fruit. The Wagener shows the effect of fertilization (Fig. 2) earlier than the McIntosh as indicated by the measurable increase in size.

The initial effect of fertilization manifests itself in an impetus given to further cell division (Fig. 5). An examination of Fig. 2 shows that the radius of the fruit suddenly begins to increase rapidly five to six days after pollination.

A study of the early developing fruits indicates that these cell divisions are general and diffuse, and that they are periclinal in orientation. The potential fruit, until postfertilization growth alters it, is somewhat ellipsoidal without a cavity at the pedicel end. There is an apical depression or basin within and around which were earlier located the free floral parts. Cell multiplication continues for different periods of time not only in the several regions of the same fruit but in comparable regions of the two varieties. A comparison of Regions 4 and 7 in Fig. 5 illustrates the first condition, a comparison of the carpellary regions (Region 7) in the two varieties, the second. In all cases the period of general cell division is over before the end of June. After this time occasional cell divisions occur sporadically throughout the regions but further increase in diameter is essentially due to cell enlargement. As with cell division, cell enlargement neither begins nor ends abruptly. The grand period of cell enlargement differs in the various regions of the same fruit and in comparable regions of the two varieties (Fig. 5). During this period, the expansion of the cell in the three planes changes the shape of the fruit from ellipsoidal to subspherical. If the enlargement of the cells in all planes were equal and no other factors entered in, a median transverse section would have the outline of a perfect circle. This period of cell enlargement comes to a gradual end in the latter part of July (Fig. 5). As this cell enlargement slows down, there occurs a rapid increase in the size of the intercellular spaces, as evidenced by the upper flattened portions of the curves in Fig. 5. From this time on further increase in volume is primarily due to this phenomenon.

If this phenomenon were equally effective in all planes and in all regions the resulting mature fruit would be the continued expansion of the sphere, and a median transverse section would merely outline a larger circle than one of an earlier date.

One of the characteristics of the Wagener fruit is its angulation, whereas the McIntosh is only faintly angled. This angulation is caused primarily by *(a)* the original degree of fluting in the outline pattern of the primary vascular bundles (4), and *(b)* a localization of growth. After the period of general cell division, the cambia of the sepal and petal bundles become active in cutting off parenchyma cells that are added to the ground tissue in Regions 5 and 6. These cells enlarge and are oriented in a radial direction. Since the cambia of these bundles in the Wagener are more active than in the McIntosh, the original angulation is accentuated. Thus there is impressed on the fundamental pattern a localization of growth that changes the shape during ontogeny.

Cells at the apex of the pedicel and around the basin are laid down during the period of cell division. As the fruit develops and the cells of these regions enlarge in the three planes, the tissue grows down and out to form the conical cavity around the pedicel, up and out to form the enlarged basin. The cavity and basin are, therefore, in considerable measure at least, the results of the mechanics of cell enlargement.

Ultimate cell size and shape are the same in comparable regions of the two varieties. The duration of cell division varies with the region and with the variety. Therefore, final regional differences between the varieties must be due to differences in cell number.

The cellular pattern of development in the apple fruit, as portrayed in the McIntosh and Wagener varieties, is laid down early in ontogeny. Later, through the operation of different physiological factors, there is added to this fundamental pattern those differences of persistence, rates, and directions of localized growth in specific tissues. The integrated activities of cell division and cell enlargement and these localized growths together with the development of an intercellular system, result in the final shape and size of the mature fruit.

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FURTHER STUDIES ON CHANGES OF DIRECTION IN THE MAJOR COIL OF THE CHROMONEMA OF *TRILLIUM ERECTUM* L.[1]

By G. B. Wilson[2] and Isabel Hutcheson[3]

Abstract

A detailed study of the direction of chromonema coiling at first meiotic metaphase and anaphase in *Trillium erectum* L. has shown that reversals in direction of coiling occur in the attachment region, at chiasmata, and in other regions. They occur with random frequency at the attachment. There is an average of two reversals at each "effective" chiasma. In other regions reversals were observed with a frequency of one in 17 gyres.

The results indicate that there is no inherent pattern determining the direction of coiling and that reversals are effected by various interrupting mechanisms.

Introduction

Any mechanism postulated to explain spiralization must meet the test of whether or not the spiral it would produce would have a directional pattern in agreement with that observed. It is, therefore, an essential part of the general problem of spiralization to determine the coiling pattern in as wide a variety of organisms as possible. So far the most detailed studies have been made on *Tradescantia*, by Nebel (9), Nebel and Ruttle (12), and Sax and Humphrey (14), on *Fritillaria*, by Darlington (1, 2, 3), and on *Trillium*, by Matsuura (7 and 8), Huskins and Smith (5), and Huskins and Wilson (6). Unfortunately the results of these studies are not in complete agreement. Darlington has stated that the arms of a chromosome almost always coil in opposite directions whereas most other workers have reported either that the direction of coiling is random or that it tends to be the same in both arms. Darlington and his co-workers have so rarely found reversals within a chromosome arm that they are inclined to consider them as relatively unimportant to general theory (16). On the other hand, workers on *Trillium* (especially Matsuura, and Huskins and his co-workers) have found many intrabrachial reversals in the major coil of meiosis. In *Tradescantia* and *Rhoeo* (12 and 13), the number of reversals per chromosome is apparently small. The present investigation has been undertaken in the hope of finding at least a partial solution to the problem of the frequency and distribution of reversals by a more detailed study of the major coil of *Trillium erectum* L. than has previously been made.

From their studies on synaptic, desynaptic, and asynaptic *Trillium erectum*, Huskins and Wilson (6) concluded that reversals in the major coil were probably of random occurrence at the attachment and at chiasmata and that, in addition, reversals could occur at any point along the chromosome with a

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[1] *Manuscript received June 19, 1941.*
Contribution from the Department of Genetics, McGill University, Montreal, Que.
[2] *Research Associate, McGill University.*
[3] *National Research Council Student.*

frequency proportional to the number of gyres. They based their conclusion on an analysis of the total number of reversals at first anaphase at which stage it is easiest to trace the coils throughout the length of the chromonema. In the present study, observations have been made at both first metaphase and anaphase.

Materials and Methods

Most of the data on synaptic material presented in this paper have been obtained from a single slide that contained both first metaphase and anaphase stages of *Trillium erectum*. This slide was chosen because of the relative ease with which all four strands could be distinguished at metaphase (Plate I). Data on desynaptic material have been obtained from the slide used by Huskins and Wilson (6).

Fixation was in 2BD and staining in crystal violet as described by Huskins and Smith (5). Observations were made with a Zeiss 1.5 mm., 1.3 N.A. objective, combined with 7× and 15× oculars. A 3 mm., 1.4 N.A. objective and 7× ocular were used when photomicrographs were taken.

In order to avoid the possibility of confusion several of the terms as they are used herein will be defined.

Interbrachial reversals. Changes in the direction of coiling that occur in the attachment region.

Intrabrachial reversals. Changes in the direction of coiling that occur within a chromosome arm.

Adventitious reversals. Those intrabrachial changes of direction not directly due to the exchange of partners at a chiasma.

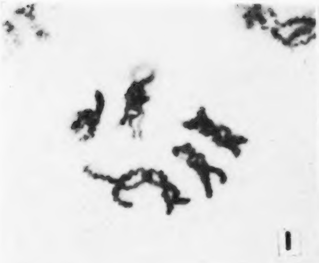
True chiasma frequency. The total number of chiasmata per complement at first metaphase, determined from preparations in which all four strands can be distinguished clearly.

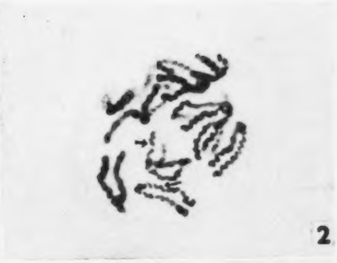
Effective chiasma frequency. The number of chiasmata per complement at first metaphase when those lying between the attachment and the proximal gyre are deducted and pairs of chiasmata separated by less than one gyre are counted as one chiasma (see also (15)). This distinction is based on the assumption, at least partly justified by observations, that any effect that chiasmata may have on coiling is purely mechanical and that two chiasmata if close together behave as a unit in this regard.

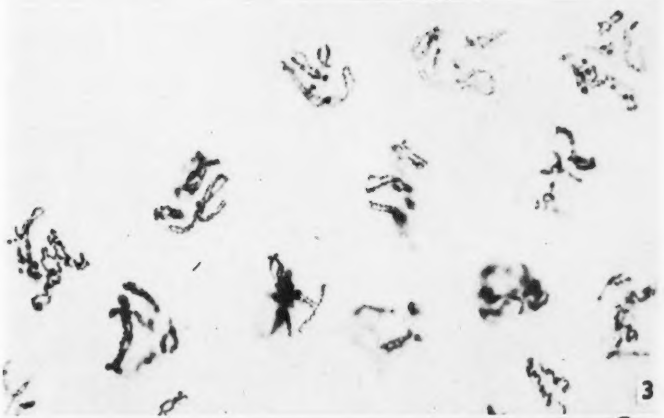
"Sister" strands. The two chromatids that are most closely paired at metaphase; these are doubtless genetic sister strands before movement of chiasmata, but not necessarily afterwards.

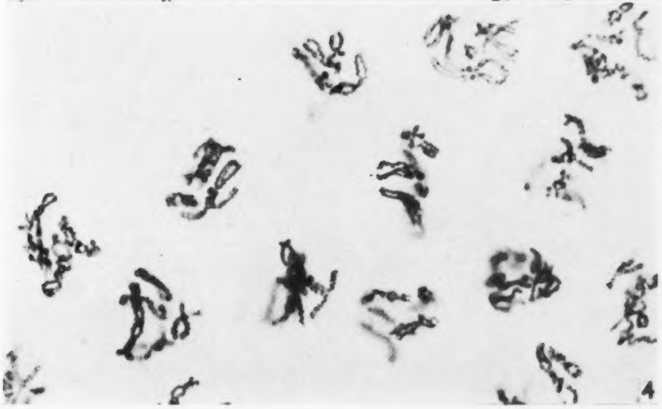
Chiasma region. The region in the vicinity of a chiasma in which the four strands are drawn out of their usual paired arrangement by the exchange of partners.

PLATE I









EXPLANATION OF FIGURES

FIG. 1. *First metaphase. Coiling is nearly completed. 750×.*

FIG. 2. *First anaphase. Note reversals in direction. 750×.*

FIGS. 3 AND 4. *Two foci of the same region showing the different stages in the development of the major coil. Coiling is just beginning in the cell at the extreme left. ca. 550×.*

Observations

(1) *Frequency of Reversals at the Attachment Region*

Huskins and Wilson (6) assumed that reversals in the direction of coiling occurred with random frequency at the attachment. They based this conclusion largely on the results obtained from asynaptic material since in this the chromatids are separated through the attachment region and there are no chiasmata to complicate the situation. Dr. A. W. S. Hunter (unpublished data) had previously found that in this material 18 out of 41 chromatids reversed direction at the attachment. While it is reasonable to assume that the effect of the attachment on direction of coiling would be similar in synaptic and asynaptic materials, this has not previously been established.

In synaptic material the effect on coiling of the attachment alone cannot readily be ascertained since the majority of bivalents have chiasmata proximal to the first gyre and they themselves have an effect on the direction of coiling. For this reason the present study has been confined to a determination of the effect of the "attachment region" on coiling direction. The "attachment region" may consist of the attachment alone, or it may also include one or two chiasmata.

A second difficulty encountered in synaptic material is that in the majority of cases the chromatids cannot be traced through the attachment. When "sister" chromatids are both coiling in the same direction on at least one side

TABLE I

AN ANALYSIS OF THE FREQUENCY OF REVERSALS IN THE ATTACHMENT REGION

Analysis	Number of reversals	
Dyad	0 or 2	1
First metaphase		
One chiasma	15	11
Two chiasmata	18	14
First anaphase	45	55
Total	78	80
Chromatid	0	1
Synaptic		
First metaphase		
No chiasmata	8	8
One chiasma	30	22
Second anaphase	14	12
Subtotals	52	42
Desynaptic	54	46
Asynaptic (Hunter)	23	18
Totals	129	106

of the attachment region there is no difficulty in making an accurate count of chromatid reversals since it makes no difference in this case whether the strands can be traced independently through the attachment or not. However, when the strands associated at the attachment become separated by chiasmata and coil in opposite directions to each other on both sides of the attachment it is impossible to determine whether no reversals or two reversals have occurred if the strands cannot be traced independently (e.g., a dyad or half-bivalent, $\frac{R}{L}\ \mathrm{o}\ \frac{R}{L}$, may actually have either 0 reversals or 2). On a random basis dyads with 0 reversals, 1 reversal, and 2 reversals would occur in a ratio of 1 : 2 : 1. Since it is not always possible to separate the 0 and 2 reversal classes they will be grouped together and tested for equality with the reversal class.

The frequency of reversals at the attachment region is given in Table I, and it was found that 78 dyads had either 0 or 2 reversals, and 80 dyads had one.

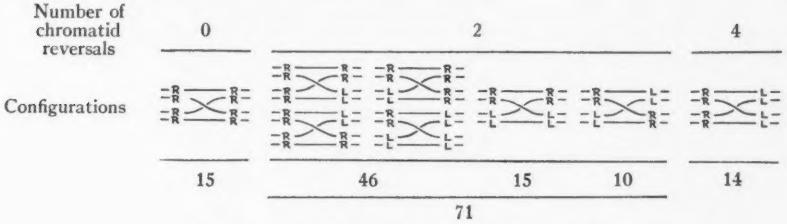
Unless the individual chromatids can be traced through the attachment region analyses cannot be made in terms of single chromatids at metaphase for bivalents with chiasmata between the attachment and the first gyre in both arms nor for dyads at first anaphase. But even if the strands cannot be traced through the attachment, since "sister" strands coil together in normal synaptic material except in the neighbourhood of chiasmata, chromatid analyses of coiling can be made when there is no chiasma between the attachment and the first gyre on either or both sides of it. The chromatid analyses given in Table I show that 42 chromatids had reversals at the attachment region and 52 did not. This number is well within the limits of random expectation ($\chi^2 = 1.06$, $p = 0.3 - 0.5$).

In desynaptic material the chiasmata are mostly resolved before coiling begins and the relation of the attachment alone to coiling direction can therefore be analysed. Furthermore, the chromatids are well separated at the attachment and can be traced individually through it with certainty. Of the 100 chromatids studied 46 had a reversal at the attachment and 54 did not ($\chi^2 = 0.64$, $p = 0.6$).

Random expectation is therefore realized in all three tests.

(2) *Frequency of Reversals at Chiasmata*

In normal synaptic *Trillium erectum* a bivalent consists of two pairs of chromatids with the direction of coiling random between the pairs but the same within each except near chiasmata; there three of the four chromatids may coil in one direction and one in the other (5, 17, and Section 3). When homologues are coiled in opposite directions, the exchange of partners at chiasmata will result in reversals of coiling. In addition, adventitious reversals may occur within the chiasma region where all four strands are separate for a short distance. Combining these two sources of reversals, eight configurations with respect to direction of coiling may occur at chiasmata (Text-fig. 1). If each of the four strands in a chiasma region is potentially capable of reversing direction with random frequency these configurations may be expected in equal numbers. These configurations represent 0,2,2,2,2,2,2 and 4 chromatid reversals, respectively, so that 0, 2, and 4 change classes are expected in a ratio of 1 : 6 : 1. A sample of 100 chiasmata were analysed and the results are presented in Text-fig. 1. The numbers found (15 : 71 : 14) fit the expected ratio ($\chi^2 = 0.89$, $p = 0.5 - 0.7$).



TEXT-FIG. 1. *Coiling configurations at chiasmata.*

(3) *Frequency of Reversals in Other Regions*

Huskins and Wilson (6) found a certain number of intrabrachial changes in direction at first anaphase in synaptic material which statistical analysis indicated were not associated with chiasmata. These were found to have a frequency proportional to chromonema length or gyre number. This analysis was supported by direct observations on asynaptic material where, also, intrabrachial changes were found to occur. In the present analysis first metaphase chromatids have been studied in detail in regions lying between successive chiasmata not less than four gyres apart, and in regions beyond the most distal chiasma. The numbers of changes occurring in these two regions were found to be similar, there being one change in 17.2 gyres in the former group and one change in 17.7 gyres in the latter.

To test in detail the conclusion of Wilson and Huskins (17) that the direction of coiling of the two pairs of chromatids of a bivalent is random with respect to each other, while the "sister" chromatids constituting a pair coil jointly, an analysis has been made, gyre by gyre, of the direction of coiling within and between the pairs of chromatids constituting a bivalent. The "between pairs" analysis showed 94 gyres in the same direction and 104 in the opposite direction. No sister chromatids were found to coil in opposite directions in those regions unaffected by chiasmata.

(4) *Frequency of Intrabrachial Reversals at First Anaphase*

First anaphase chromosomes have been analysed to determine the coiling pattern in whole complements of 20 chromatids. In this analysis the relationship between sister strands is disregarded. The observations were made on 10 cells from the same slide that was used for the first metaphase analyses. The mean number of intrabrachial reversals per complement was found to be 35.9 ± 1.9 and the mean number of gyres 209.5 ± 1.9 (Table II). Though not pertinent to the present problem, it is of interest to note that there were 1126 dextral and 1069 sinistral gyres. The difference is not a significant deviation from equality.

TABLE II

NUMBER OF INTRABRACHIAL REVERSALS AND NUMBER OF GYRES AT FIRST ANAPHASE

Cell	Number intrabrachial reversals	Number gyres
1	29	208
2	26	197
3	41	219
4	36	214
5	41	209
6	35	212
7	32	205
8	37	203
9	49	214
10	33	214
	359	2095
Mean	35.9 ± 1.9	209.5 ± 1.9

Discussion

A comparison of the behaviour of synaptic, desynaptic, and asynaptic materials has shown that, in all three, reversals take place at random in the attachment region. In the synaptic material one or two chiasmata are usually associated with the attachment, whereas, in the other two, no chiasmata are involved. Since there is such close agreement among the materials with regard to the effect of the "attachment region", it seems reasonable to assume that the attachment plus one or two associated chiasmata has the same effect on coiling as the attachment alone.

At chiasmata it has been shown that 0, 2, and 4 chromatids change in a ratio not statistically significant from the expected ratio of 1 : 6 : 1, indicating that adventitious reversals and those due to exchange of partner occur in equal numbers. Since the 0 and 4 change classes are equal, there must be an average of 2 reversals to a chiasma. It is, however, impossible to differentiate between reversals brought about by change of partner at a chiasma and those that occur through undefined causes in the unpaired region adjacent to it.

No significant difference was found between the number of adventitious reversals in regions between chiasmata and the number in regions distal to the last chiasma. In both cases changes occurred approximately once in

17 gyres. The undefined causes producing adventitious reversals must include the meeting of regions that have independently begun to coil in opposite directions, as they often clearly appear to do in *Trillium*. This may be a large factor in determining the reversals at chiasmata since these are obviously points of interference in the coiling process.

In their study on the direction of coiling, Huskins and Wilson (6) subdivided the total number of changes per nucleus observed at first anaphase into several groups. These groups were then used to reconstruct the hypothetical first metaphase bivalent in which changes in direction of coiling had earlier occurred. In the present study changes have been observed at first metaphase. The first anaphase configurations that would result from these have been predicted and compared with those observed at this later stage (Table III). There is close agreement between the calculated and the observed number of changes.

TABLE III

FREQUENCY OF INTRABRACHIAL REVERSALS AT FIRST ANAPHASE

Observed		Hypothetical				
Intrabrachial reversals per cell	Gyres	Intrabrachial reversals			Gyres*	
		Within chiasma regions**	In other regions	Total	Within chiasma regions	At other loci
35.9 ± 1.9	209.5 ± 1.9	31	5	36	125	85
Chiasma frequency						
True	Effective					
24.36 ± 0.58	15.6 ± 0.4					

* *It has been assumed on the basis of the observations that each chiasma involves approximately eight gyres (two gyres per strand).*

** *Computed from the effective chiasma frequency.*

Conclusions

While some details of the problem of the direction of coiling are yet to be investigated there seems little doubt from the results presented here together with those of Matsuura (8) and Huskins and Wilson (6) that the distribution of reversals in the direction of coiling is incompatible with torsion theories of spiralization. The fact that reversals occur with random frequency at the attachment region and at chiasmata and with a frequency proportional to length elsewhere indicates that there is no inherent structural pattern within the chromonema that determines direction of coiling or causes reversals.

Acknowledgments

The authors wish to take this opportunity of thanking Professor C. L. Huskins and Dr. A. G. Steinberg for their helpful suggestions and criticisms during the course of this work. The junior author also wishes to acknowledge the financial aid of the National Research Council of Canada.

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VEGETATIVE PROPAGATION OF CONIFERS

X. EFFECTS OF SEASON OF COLLECTION AND PROPAGATION MEDIA ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By J. L. Farrar[2] and N. H. Grace[3]

Abstract

Twenty-four collections of Norway spruce cuttings were taken, seven about the time new growth was forming, six at semimonthly intervals from July to September, four during October, and seven at monthly intervals during the winter to April, and were propagated in outdoor frames in several media. The proportion of cuttings rooting in sand was low for the summer collections but reached 80% or higher for collections made in September and October. The addition of sedge peat effected rooting of 90% in collections taken throughout summer and autumn, and increased the number of roots, length of root, and the development of new growth. Sphagnum peat added to the sand was also slightly beneficial but, for propagation, much inferior in effect to the sedge type of peat. Varying the proportion of peat or the texture of the sand had no significant effect except on the length of root, which was greater in those media rich in peat. Cuttings stored over winter or taken in spring did not respond well.

Previous communications by the authors and others have dealt with the vegetative propagation of conifers (1–13). A recent article summarized the effects of chemical treatments on the outdoor propagation of Norway spruce cuttings (11). In this communication is reported the result of a series of experiments directed to the outdoor propagation of Norway spruce cuttings taken at various seasons and propagated in different media.

Experimental

This investigation of the effects of developmental stage, for convenience designated period of collection, and media on propagation involved 10 experiments and approximately 10,000 cuttings; only six of the experiments will be discussed in detail, the results from the others will merely be summarized. Most of the experiments were of factorial design permitting consideration of other factors such as chemical treatments or type of cutting in conjunction with effects of media and collection. The general methods of collecting and planting the cuttings have been described (11, 12).

Three different types of media were used, namely, sand, sand mixed with an imported peat of sphagnum origin, and sand mixed with a domestic, well decomposed peat of sedge origin* (4, 12). Data concerning chemical analyses of the two different peats are given in Table I. It is apparent that the sedge type of peat has a substantially greater content of both acid soluble and

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* *The sphagnum peat, from Sweden, was obtained through a local horticultural supply house; the sedge peat was obtained directly from Alfred, Ont.*

TABLE I

CHEMICAL ANALYSES OF SEDGE AND SPHAGNUM PEAT

	Composition, %			
	Sedge peat		Sphagnum peat	
Loss on ignition	80.6		95.0	
Containing nitrogen		2.12		0.94
Mineral matter insoluble in acid	13.4		3.5	
Mineral matter soluble in acid	6.0		1.5	
Containing: lime		2.16		0.40
potash		0.06		0.09
phosphoric acid		0.23		0.23
Acidity (pH)	5.6		4.0	

The authors are indebted to the Division of Chemistry, Central Experimental Farm, for the analyses.

insoluble minerals and more nitrogen; it is also less acidic. Though the chemical properties of these peats are different, their physical characteristics are essentially similar. The usual moisture content of media containing one-third peat by volume was about 14% of the oven-dry weight whereas that of sand alone was about 4%. The sand media and sand–peat mixtures involved the use of two different types of sand, one relatively coarse, the other fine; screen analyses of these sands are given in Table II. Equal proportions of these sands were used in the sand media of Experiments 1, 2, and 3 and in the peat mixtures of Experiments 1, 2, 3, 5, and 6 and several proportions of each as described under Experiment 4; fine sand alone mixed with an equal amount of peat was also used for Experiments 5 and 6.

TABLE II

SCREEN ANALYSES OF SANDS USED AS MEDIA

Mesh, openings to the inch	Coarse sand		Fine sand	
	Retained, %	Passed, %	Retained, %	Passed, %
4	1.0	99.0	1.3	98.7
8	4.4	95.6	2.9	97.1
10	12.8	87.2	4.2	95.8
20	56.6	43.4	8.9	91.1
35	92.5	7.5	28.6	71.4
65	99.2	0.8	77.5	22.5
100	99.7	0.3	90.8	9.2

Experiment 1

Cuttings were collected at semimonthly intervals during July and August, 1939, and planted in three media. These were sand, sand mixed with one-third by volume of sphagnum peat, and sand with one-third by volume of sedge peat.

Experiment 2

In this experiment the semimonthly collections of Experiment 1 were continued throughout September and October. Media were reduced to two by eliminating the sphagnum peat mixture.

Experiment 3

Cuttings were collected October 29, 1939, and planted in the two peat media described under Experiment 1.

Experiment 4

Cuttings were collected October 29, 1939, and planted in 28 different media in compartments 1 × 3.5 ft. in size. Sand was mixed with sphagnum and sedge peat, each separately, in proportions by volume of eight sand to one, two, four, and eight of peat. The mixture of eight sand to four peat was identical with the media used in the preceding experiments. Three different grades of sand were used to make the various media; these were the two described in Table II, and a mixture of the two in equal proportions. In addition to 24 mixtures of sand and peat there were four sand media—one volume coarse sand with one, two, and four volumes of fine sand, and fine sand alone.

Experiment 5

The branches were collected approximately every four weeks starting November 17, 1939, with the last of the seven collections April 23, 1941. Cuttings were made and heeled in in flats in a sand–peat mixture and kept outdoors protected with a mulch of straw. In May the cuttings were planted in two media, the sedge peat mixture of Experiments 1, 2, and 3 and fine sand with an equal volume of sedge peat. At the time of the last collection cuttings were also taken from branches of the first collection, which had been stored outside during the winter.

Experiment 6

Collections were made June 6, 1940, after the new shoots were about one inch in length and June 27 when elongation of new growth on the lateral branches had ceased. Cuttings of this second collection were entirely of 1940 growth. Both collections were propagated in the media of Experiment 5.

A group of experiments was started in the spring of 1939. These involved collections about two weeks prior to the emergence of new growth, just before emergence, during the opening of the buds and after the shoots had appeared. In addition, there were collections in which the cuttings were entirely of 1939 growth. The cuttings for all these experiments were propagated in sand only.

It has also been possible to develop some additional criteria from the results of a greenhouse experiment already reported by Farrar (3) and Deuber and Farrar (2). The original papers dealt chiefly with effects on rooting; the additional results now considered refer to effects of time of collection on the development of new growth by the cuttings and the numbers and lengths of root per rooted cutting.

The cuttings of Experiments 1 to 6 were removed for observation in September, 1940. Record was made of the number of cuttings surviving, callused, rooted, and the number and length of roots per group of cuttings. The number and length of roots per rooted cutting and the mean root length were calculated. Data were recorded separately for cuttings with and without new growth. The number of new growth shoots per group of cuttings and the aggregate length of the longest shoot were determined for rooted and not rooted cuttings. Statistical treatment of similar data has been described in earlier articles (3, 5–13).

Results

Table III indicates the effects of the propagation medium and season of collection on responses of Norway spruce cuttings. The data are averages over other experimental factors such as chemical treatment, type of cutting, and proportions of peat and sand in the medium when these factors were without significant effects.

It is apparent from the data of the table that cuttings collected between mid-July and late October and propagated in a sedge peat medium responded favourably. Those planted in sand or sphagnum peat media, or in a sedge peat medium at other times of the year did not respond so well.

The season of collection had little effect on the rooting of cuttings planted in sedge peat from mid-July to late October. On the average, over 90% of the cuttings were rooted each with four to five roots averaging about 5 cm. in length. The best individual group of 10 cuttings is illustrated in Fig. 1. The percentages of winter and spring collections rooting was not as high and the average number of roots was substantially lower. However, the mean root length did not differ greatly in the winter collections, though it was much shorter in the spring collection (Experiment 6). These cuttings were, of course, in the beds for a shorter period than the previous collection. In both the sand and sand–sphagnum-peat media there were many living cuttings that failed to root and mortality was markedly greater than in the sedge peat media. The proportion of living non-rooted cuttings was particularly high at the period of low rooting and survival was also low at these periods.

The proportion of cuttings with new growth was approximately the same among rooted cuttings and those living but not rooted and it is apparent that the presence of new growth on a cutting was no indication that it was necessarily rooted. Under the more favourable conditions of media and collection (plantings in sedge peat media and summer collections) most of the rooted cuttings bore new growth, while under less favourable conditions most of the rooted cuttings lacked such growth. (In the collection of June 6, Experiment 6, all cuttings had new growth from the beginning.) The effect of media and season of collection on the production of new growth shoots was similar to the effects on survival and rooting.

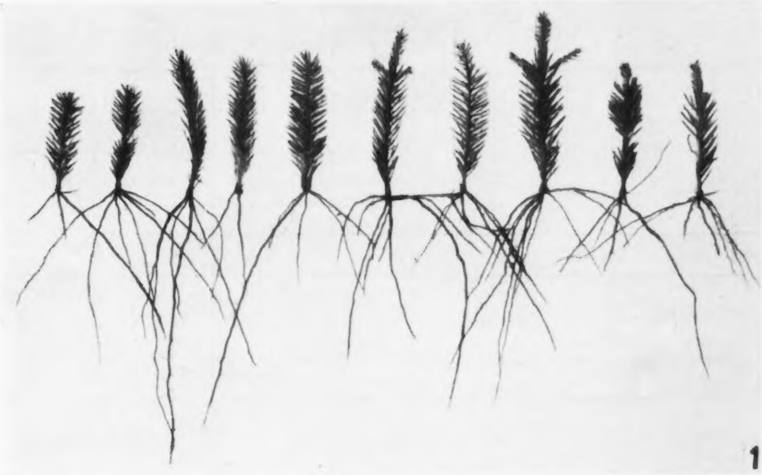

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FIG. 1. *Norway spruce cuttings propagated outdoors in a sedge peat medium; the best individual group of 10 cuttings. (Background ruled in inches.)*

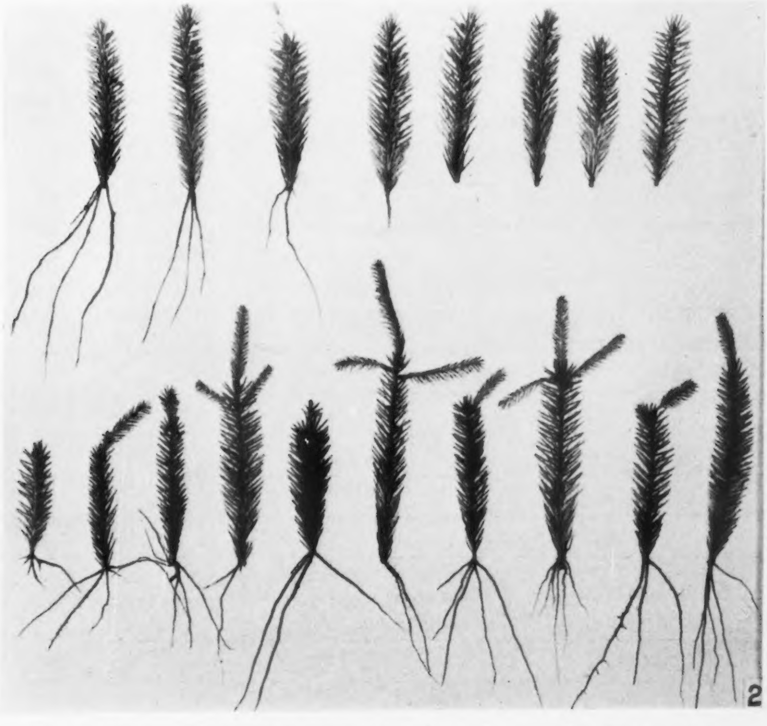

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FIG. 2. *Norway spruce cuttings propagated outdoors in peat media. Upper: sphagnum peat. Lower: sedge peat. (Background ruled in inches.)*

A large proportion of the cuttings planted in sedge peat media during the summer produced new growth shoots the following spring. In sand only, few cuttings produced new growth but the proportion increased markedly with the later collections. Most of the cuttings with new growth bore only one shoot but the summer collections in sedge peat media resulted in a greater number and length of shoots than was found for the other two types of media or other times of collection. In the first collection of Experiment 6 new growth was produced while the cutting was still on the tree, and on the average, there were finally two shoots per cutting.

The data on numbers and lengths of roots and the proportion of surviving cuttings that rooted were analysed separately for cuttings with and without new growth. It was found that the results were essentially similar for both classes of cuttings. Data on numbers and lengths of new growth shoots were analysed separately for cuttings with and without roots and it was again shown that the results for both classes of cutting were essentially similar.

An outstanding feature of the results is the difference between sphagnum and sedge peats. Reference to the data for Experiments 3 and 4, taken at probably the optimum season for the outdoor propagation of Norway spruce cuttings, shows that in sedge peat the percentage rooting and length of root per rooted cutting and the number with new growth was approximately twice or more that obtained in sphagnum peat, and there were more and longer shoots. Typical differences between the effects of the two peat media on rooting and development of new growth are illustrated in Fig. 2.

The different proportions of peat used in Experiment 4 affected only the length of root per rooted cutting. There was a greater length of root associated with an increasing proportion of peat. Likewise the different proportions of sand made no significant difference to any of the factors used as criteria. The poor results in sand in Experiment 4 as compared with a similar collection in Experiment 2 may be attributed to deficient water supply. In Experiment 4 the compartments of sand were interspersed at random with compartments of peat media, which require less water; since the bed was watered as a whole the sand did not receive an adequate supply.

It is apparent from the data for Experiment 5 that cuttings made and planted in April (Collection 7) gave better survival and rooting than those made in November, which are, again, better than cuttings taken at the same time but stored over winter on the branch. However, about 82% of the surviving cuttings of all three of these collections were rooted.

Spring collections made in 1939, and planted outdoors in sand, resulted in rooting of 10 to 25% of the cuttings taken in May about two weeks prior to emergence of new growth; this proportion rose to about 40% for those cuttings taken four days prior to emergence of new growth, and 75% for those in which new growth was breaking. About 64% of cuttings of 1938 wood bearing shoots ranging in length from 0.5 to 1 cm. rooted (11), and 45% of those with shoots from 1 to 2 cm. in length. The mortality of cuttings con-

TABLE III

RESPONSES OF NORWAY SPRUCE CUTTINGS TO PROPAGATION MEDIUM AND SEASON OF COLLECTION (CUTTINGS WERE REMOVED SEPTEMBER, 1940)

Expt. No.	Collection date	Criterion								
		Number of surviving cuttings, %			Number of rooted cuttings, %			Number of cuttings rooted as percentage of those surviving		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
	1939									
1	July 12	31	78	96	26	70	90	84	90	94
	July 26	31	94	100	16	76	98	52	81	98
	Aug. 9	13	78	100	8	56	98	62	72	98
	Aug. 23	15	62	98	5	32	82	33	52	84
2	Sept. 15	78		98	56		90	72		92
	Sept. 29	97		99	86		97	89		98
	Oct. 13	92		97	81		96	89		99
	Oct. 27	100		99	95		98	95		99
3	Oct. 29		65	94		40	90		61	96
4	Oct. 29	75	82	97	54	56	93	73	69	96
5	Nov. 17			57			47			82
	Nov. 17†			40			33			82
	Dec. 11			67			49			74
	1940									
	Jan. 8			53			33			61
	Feb. 2			46			29			64
	Feb. 21			52			38			73
	Mar. 27			70			44			63
	April 23			95			78			83
6	June 6			74			37			49
	June 27			52			26			49

Expt. No.	Collection date	Criterion								
		Number of roots per rooted cutting			Length of roots per rooted cutting, mm.			Mean root length, mm.		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
	1939									
1	July 12	4.0	4.3	5.3	129	237	276	32	55	52
	July 26	2.9	3.8	4.9	53	143	248	18	38	50
	Aug. 9	3.5	3.3	4.3	90	114	234	26	35	54
	Aug. 23	2.6	2.7	4.2	17	33	109	6	12	26
2	Sept. 15	3.7		4.4	80		172	22		39
	Sept. 29	3.7		4.4	81		206	22		47
	Oct. 13	4.0		5.0	86		268	22		54
	Oct. 27	4.6		4.6	164		287	35		62
3	Oct. 29		3.3	4.2		127	225		39	54
4	Oct. 29	3.0	3.1	4.3	76	130	260	26	42	60
5	Nov. 17			3.0			145			48
	Nov. 17†			2.5			145			59
	Dec. 11			2.8			133			47
	1940									
	Jan. 8			1.9			103			55
	Feb. 2			2.1			99			47
	Feb. 21			2.7			133			49
	Mar. 27			2.9			153			53
	April 23			3.3			178			55
6	June 6			2.2			28			13
	June 27			2.1			14			7

† *The cuttings of this collection were made April 23, 1940, from branches held outside in storage from November 17, 1939.*

TABLE III—*Concluded*

RESPONSES OF NORWAY SPRUCE CUTTINGS TO PROPAGATION MEDIUM AND SEASON OF COLLECTION (CUTTINGS WERE REMOVED SEPTEMBER, 1940)—*Concluded*

Expt. No.	Collection date	Criterion								
		Number of cuttings with new growth, %			Number of cuttings with new growth as a percentage of those surviving			Number of rooted cuttings with new growth as a percentage of those rooted		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
	1939									
1	July 12	2	4	64	6	5	67	8	6	69
	July 26	0	0	54	0	0	54	0	0	55
	Aug. 9	0	2	38	0	3	38	0	4	37
	Aug. 23	5	4	58	33	6	59	4	12	63
2	Sept. 15	23		61	29		62	30		62
	Sept. 29	57		66	59		67	57		68
	Oct. 13	39		68	42		70	46		71
	Oct. 27	60		73	60		74	60		73
3	Oct. 29		12	70		19	75		24	75
4	Oct. 29	31	28	75	42	34	77	43	34	77
5	Nov. 17			7			12			12
	Nov. 17†			3			8			6
	Dec. 11			15			23			25
	1940									
	Jan. 8			7			12			13
	Feb. 2			4			9			*
	Feb. 21			14			27			25
	Mar. 27			10			14			17
	April 23			37			39			43
6	June 6			74			100			100
	June 27			0			0			0

Expt. No.	Collection date	Criterion								
		Number of rooted cuttings with new growth as a percentage of those with new growth			Number of shoots per cutting with new shoots			Mean length of longest shoot on each cutting with new shoots, mm.		
		Medium								
		Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat	Sand	Sphagnum peat	Sedge peat
	1939									
1	July 12	*	*	97	*	*	1.59	*	*	22
	July 26	*	*	100	*	*	1.33	*	*	18
	Aug. 9	*	*	95	*	*	1.32	*	*	18
	Aug. 23	*	*	90	*	*	1.21	*	*	25
2	Sept. 15	74		92	1.13		1.38	18		29
	Sept. 29	86		100	1.28		1.39	20		25
	Oct. 13	95		100	1.10		1.34	17		24
	Oct. 27	95		99	1.22		1.45	20		33
3	Oct. 29		79	95		1.10	1.36		18	35
4	Oct. 29	74	69	95	1.14	1.10	1.39	16	17	27
5	Nov. 17			76			1.00			20
	Nov. 17†			62			1.12			24
	Dec. 11			81			1.24			27
	1940									
	Jan. 8			63			1.12			18
	Feb. 2			*			1.20			17
	Feb. 21			68			1.12			25
	Mar. 27			75			1.33			20
	April 23			90			1.24			22
6	June 6			49			2 06			24
	June 27			?						

† *The cuttings of this collection were made April 23, 1940, from branches held outside in storage from November 17, 1939.*

* *Meagre data.*

sisting entirely of 1939 growth planted in sand in early June was 100%. In cuttings of 1939 wood with new growth planted in sand peat in 1940, 37% rooted while the proportion of new growth cuttings rooted was 26% (Table III, Experiment 6).

Counts of the number of cuttings with new growth and the numbers and lengths of root per rooted cutting for winter collections of cuttings propagated in sand in the greenhouse at New Haven indicated marked effects from season of collection. Data were meagre for cuttings of the October collection, those of the following three months had 2.6, 4.1, and 3.2 roots per rooted cutting, respectively, and the corresponding lengths of root were 32, 121, and 94 mm. Thus, December and January collections produced more and longer roots than the one in November. Even more striking differences were revealed by the counts of new growth which were approximately 80 and 90%, respectively, for December and January collections but less than 1% for October and November collections.

Discussion

These results have demonstrated the great effect of medium on the rooting of summer and autumn collections of Norway spruce cuttings propagated outdoors, and are similar in nature to earlier results for fall and winter collections (4, 10–12). When a suitable medium such as well decomposed sedge peat is used, effects attributable to the period of collection are slight. However, when a much less suitable medium such as sand only is employed, period of collection of the cuttings may have a pronounced effect on the results.

The authors have already discussed changes in rooting response with period of collection (3, 4, 7, 9) and recently, Deuber has presented such data also for Norway spruce cuttings propagated in sand in the greenhouse (1). The present experiments indicate certain modifications of the earlier views. The percentages of rooted cuttings of new growth, taken when elongation of the lateral twigs has ceased, were low. Three weeks later about 90% of the cuttings planted in a sedge peat medium were rooted and this level was maintained until freeze-up. Cuttings planted in sand did not root well until September, from then on the rooting percentage was almost as high as in peat though numbers and lengths of roots were lower.

After the ground is frozen cuttings must either be planted in a greenhouse or stored till spring. The results of Experiment 5 indicate that the latter practice substantially reduces rooting. This may be attributed to unfavourable storage conditions and injury in transplanting. The rise in rooting from February to March could be explained in part by the shorter storage period. The decline in rooting from November to February is very similar to that observed in the greenhouse by Deuber and the authors (1, 7, 9). It seems likely that the decline in the rooting potential at this period is due to physiological changes in the tree.

Results have been variable with cuttings taken before and during the development of new growth. There is evidence that cuttings taken just as the buds are breaking root more readily than cuttings taken just before or just after this time.

These experiments indicate that bud development and rooting occur independently. However, conditions favourable to the one also favour the other.

The fact that the beneficial effect of sedge peat in sand mixtures is, within wide limits, unaffected by the proportion of peat in the texture of the sand, greatly simplifies its use in practice.

Norway spruce cuttings taken during summer or autumn can be propagated outdoors in readily constructed frames by the use of sedge peat mixed with the sand. This finding is of particular interest to tree breeders and horticulturists who may wish to propagate certain individuals of this species.

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STUDIES IN DIFFERENTIAL REACTIVITY

I. THE RATE AND DEGREE OF DIFFERENTIATION IN THE SOMATIC CHROMOSOMES OF *TRILLIUM ERECTUM* L.[1]

By G. B. Wilson[2] and E. Roger Boothroyd[3]

Abstract

By suitable treatment the somatic chromosomes of a number of plants may be shown to be longitudinally differentiated as to size and staining capacity. Certain well defined regions appear at metaphase and anaphase to be either understained or of reduced diameter or both.

Exposure to cold has been found to produce these regions in two varieties of rye and three species of *Trillium*.

The positions of these differential segments have been found to be highly specific for different chromosomes and species.

During a 96-hr. period of exposure to cold the number of chromosomes affected and of differential segments per chromosome increases in *T. erectum* L.

After cessation of treatment the chromosomes resume their normal appearance within a few hours.

Homologous chromosome pairs often differ in number, size, and position of affected regions. On the basis of data presented in this paper, these differences cannot be taken as conclusive evidence that these pairs are genetically different.

Introduction

From time to time there have appeared in the literature accounts of experimental treatments that rendered visible a differentiation of the chromosomes not otherwise observable. This differentiation has taken the form of regions of reduced diameter and staining capacity and of secondary constrictions, sometimes so numerous as to give the chromosomes a chromomeric appearance. The former may be of considerable length and may appear either within or at the ends of chromosome arms.

Kagawa (9, 10) and Ellenhorn (4) have shown that pretreatment of *Triticum* root tips with chloral hydrate before fixation renders visible certain specifically localized secondary constrictions that cannot be seen without such pretreatment. Similar results have also been obtained in the somatic chromosomes of *Trillium smallii* Maxim. and *T. Tschonskii* Maxim. (8).

Geitler (7) observed "chromomeres" in the somatic metaphase chromosomes of *Crepis capillaris* Wallr. after fixation with the Flemming–Benda mixture. Similar results were obtained, according to Shmargon (12) in *Allium cepa* L. by growing the plants in sawdust, and fixing with Champy's fluid. One chromosome contained over 20 "chromomeres" well differentiated in size.

Kakhidze (11) confirmed Geitler's observations in *Crepis*, finding the metaphase chromosomes uniformly divided into "chromomeres". When the roots

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were precooled, however, secondary articulations that were apparently quite distinct from the "chromomeres" appeared in each arm.

Shmargon (12) observed a "chromomeric structure" in the mitotic chromosomes of *Secale cereale* L. after precooling the material (24 hr. at 0° C.) and using Levitsky's "strong platinic formalin" and Champy's fixatives. He believes these "compound chromomeres" to be formed by fusion of the "ultimate chromomeres".

Darlington and La Cour (2, 3) found understained regions of reduced diameter, and highly specific as to locality, in mitotic metaphase and anaphase chromosomes of *Paris* and *Trillium* subjected to cold treatment for several days. They called this phenomenon "differential reactivity". The same condition was obtained by Coleman (1) in the root tip chromosomes of *Trillium grandiflorum* Salisb. and in this laboratory, in the microspore chromosomes of *T. erectum* (13, Fig. 17).

In arctic species of *Ranunculus* and grasses, Flovik (5, 6) found an exceptionally high number of secondary constrictions in most cases. In some species, e.g., *R. pygmaeus* Wg., three *Phippsia* species, and *Arctophila fulva* Rupr., so many constrictions were found that the chromosomes had a beaded appearance similar to that observed in *Crepis* and *Secale*. The resolution of these constrictions depends to some degree on the choice of fixatives, those containing little or no acetic acid being most satisfactory.

In brief, after appropriate fixation "compound chromomeres" and/or secondary constrictions have been seen in the somatic chromosomes of *Allium*, *Crepis*, *Secale*, *Ranunculus*, and several genera of grasses. After cold treatment secondary constrictions and differential segments have been found in the somatic chromosomes of *Crepis*, *Paris*, *Fritillaria*, and *Trillium*; in *Triticum*, *Trillium smallii*, and *T. Tschonskii* secondary constrictions have also been obtained after pretreatment of the material with chloral hydrate. These conditions may all be considered as "differential reactivity" and, although proof is not yet available, it seems probable that they are all manifestations of the same phenomenon. As both mitotic and meiotic chromosomes are now known to have their chromonemata coiled at metaphase and anaphase, it may be concluded that the "chromomeric" appearance at these stages bears no more than a superficial resemblance to the chromomeric structure of early prophase.

Since such differential reactivity almost certainly indicates a basic differential pattern in the chromosomes, knowledge concerning the origin and behaviour of such regions may be of considerable importance in the elucidation of chromosome structure, behaviour, and constitution. There are many angles from which the study of differential reactivity may be investigated, eventually involving chemical methods, but it is the opinion of the authors that before such methods are employed an exhaustive study of a purely cytological nature should be made. The experiments to be described in this paper represent their initial approach to this problem. Although they are necessarily preliminary in nature and cannot justifiably be used as a basis for extensive speculations, they have brought to light several facts which, we believe, will eventually prove of considerable importance in the final elucidation of this problem.

Materials and Methods

Most of the present investigation has been based on *Trillium erectum* but some studies have also been made on *T. grandiflorum*, *T. undulatum* Willd., and *Secale cereale* var. Horton and Rosen. Root tip mitoses were studied in all cases. Cold treatment was provided by a commercial electric refrigerator that maintained a fairly constant temperature of 3° C. Root tips of all *Trillium* species were fixed in three parts absolute alcohol and one part glacial acetic acid, macerated in 45% acetic acid at 60° C., and stained with aceto-carmine. This method was also used on *Secale* but better results were obtained by fixing in La Cour's 2BD with subsequent staining by the Feulgen technique.

Observations were made with a Zeiss 1.5 mm., 1.3 N. A. objective and 7× oculars. A Zeiss 3 mm., 1.4 N. A. objective and a 7× ocular were used when photomicrographs were taken.

Observations

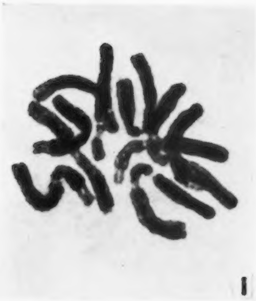
THE EFFECT OF COLD TREATMENT ON THE SOMATIC CHROMOSOMES OF RYE

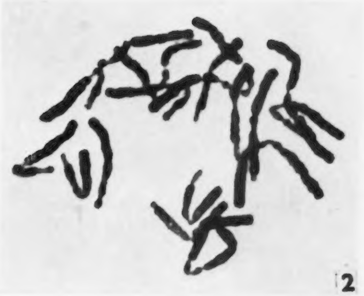
Grains of rye were germinated in sawdust and subjected to a temperature of 3° C. for 72 hr. Late prophase chromosomes were found to be beaded in appearance as described by Shmargon (12). Metaphase and anaphase chromosomes were similarly affected, but not to the same extent. The appearance of the affected regions at these stages was similar to that of the differential segments in *Trillium* (Figs. 6 and 7).

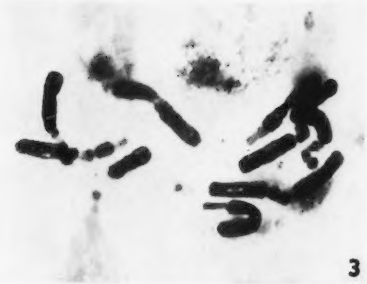
THE EFFECT OF COLD TREATMENT ON THE SOMATIC CHROMOSOMES OF *Trillium*

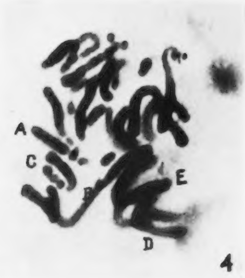
As described by Darlington and La Cour (3) exposure to cold for a sufficient length of time prevents certain regions of the metaphase and anaphase chromosomes of *Trillium* from staining normally. As a rule these regions are understained and are only about one-half the diameter of the unaffected portions of the chromosome (Figs. 1 to 5). Occasionally, however, these regions have been found to be understained but not noticeably narrower. Also, on occasion, regions much narrower than normal appear to be well stained. These conditions are, however, rare. The terminally situated differential regions in *Trillium* are usually quite long whereas interstitial ones are ordinarily very short. For the most part the interstitial segments are within the proximal third of the chromosome arm but several chromosomes, notably the *D* chromosome of *T. erectum*, *A* of *T. grandiflorum*, and *C* of *T. undulatum* (Fig. 4) characteristically have differential segments in the distal third of one arm.

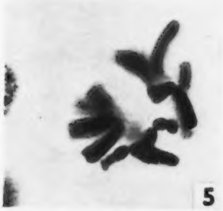
PLATE I

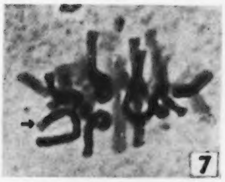


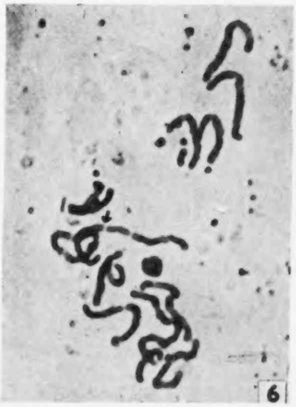












EXPLANATION OF FIGURES

FIGS. 1, 2, 3. *Root tip chromosomes of Trillium erectum* L. *showing differential segments. Figs. 1 and 3 are metaphase, Fig. 2, anaphase. ca. 700×.*

FIG. 4. *An anaphase cell from a root tip of T. undulatum* Willd. *Chromosomes A, B, and C show differential segments while D and E appear to be normal. ca. 700×.*

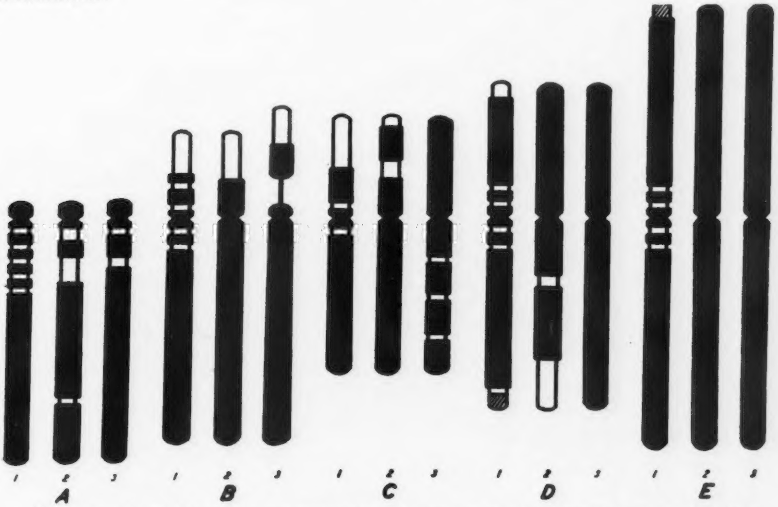
FIG. 5. *A metaphase cell from a root tip of T. grandiflorum* Salisb. *showing differential segments in the B, C, and D chromosomes. Note that chromosome E is normal in appearance. ca. 700×.*

FIG. 6. *Early metaphase from a root tip of Secale cereale* L. *var. Rosen showing an interstitial differential segment. ca. 1100×.*

FIG. 7. *A metaphase plate from a root tip of S. cereale* L. *var. Horton. Note terminal differential segment similar to those found in Trillium. ca. 1100×.*

The Specificity of the Differential Segments

Although the size of the differential segments is subject to some variation, they are highly specific as to position. Each chromosome has a characteristic pattern. The diagram in Text-fig. 1 shows the position and average size of all regions, in all three species, that have at one time or another shown the reaction. These diagrams represent all the regions found in the present experiments. Chromosomes *A* and *C* of *T. erectum*, *A*, *B*, *C*, and *D* of *T. grandiflorum*, and *A*, *B*, and *C* of *T. undulatum* have at times been found to be affected in all susceptible regions. No differential segments were found in the *E* chromosome of *T. grandiflorum* or in the *D* and *E* of *T. undulatum*.



TEXT-FIG. 1. *A diagram showing the size and position of all differential regions that have been observed in the somatic chromosomes of Trillium. (1) T. erectum* L., *(2) T. grandiflorum* Salisb., *and (3) T. undulatum,* Willd. *Cross-hatched regions appear only rarely.*

Some regions are apparently more readily affected than others. This is indicated in Table I where seven plants of *T. erectum* in which 90% or more of the chromosomes are affected have been classified to show their distribution in the various possible combinations of terminal, terminal plus interstitial, and interstitial regions. It is quite obvious from these data that the terminal regions of chromosomes *B*, *C*, and *D* are more easily affected than their interstitial segments. Part of this difference may be due to our inability to see segments that are not markedly affected, but it is unlikely that the disproportion is entirely due to this factor, since there seems to be little difficulty in discerning the subterminal region in the *D* chromosome which as a rule is less obvious than other interstitials. It has not been possible to differentiate accurately among the proximal differential segments and, therefore, not possible

TABLE I

THE DISTRIBUTION OF DIFFERENTIAL SEGMENTS IN *Trillium erectum* L.

Segments	Chromosome	Material										
		72-D_2-96	72-F_2-96	72-*M*-96	72-*O*-90	72-*P*-96	72-*Q*-96	72-*R*-96	Subtotal	72-*R*-336	72-*R*-676	Total
Normal	*A*	4	2	2	6	12	0	0	26	1	1	28
	B	1	6	0	6	1	0	0	14	0	1	15
	C	1	3	0	2	3	0	2	11	0	2	13
	D	4	2	9	4	9	4	0	32	0	0	32
	E	0	5	1	1	3	0	0	10	0	0	10
1 Terminal	*A*	0	0	0	0	0	0	0	0	0	0	0
	B	15	23	33	3	32	9	7	122	15	4	141
	C	26	39	45	35	96	22	18	281	31	13	326
	D	7	21	22	9	27	8	4	98	5	3	106
	E	0	0	0	1	0	0	0	1	0	0	1
1 Terminal + 1 interstitial	*A*	0	0	0	0	0	0	0	0	0	0	0
	B	10	14	13	9	51	10	10	117	9	14	140
	C	1	0	0	3	2	0	1	7	3	3	13
	D	9	19	11	8	29	7	7	90	15	13	118
	E	0	1	0	0	0	0	0	1	0	0	1
1 Terminal + 2 interstitial	*A*	0	0	0	0	0	0	0	0	0	0	0
	B	2	3	1	7	7	2	3	25	6	3	34
	C	0	0	0	2	0	0	0	2	0	0	2
	D	2	2	1	9	9	0	5	28	10	5	43
	E	0	0	1	1	0	0	0	2	0	0	2
1 Terminal + 3 interstitial	*A*	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	1	1	1	0	0	3	0	0	3
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	2	0	1	2	0	1	6	2	1	9
	E	0	0	0	0	0	0	0	0	0	0	0
1 Interstitial	*A*	13	23	28	6	33	6	2	111	3	6	120
	B	0	4	0	8	2	1	0	15	0	0	15
	C	0	0	0	1	0	0	0	1	0	1	2
	D	4	3	5	10	13	2	1	38	2	1	41
	E	0	4	8	4	24	4	3	47	2	3	52

TABLE I—*Concluded*

THE DISTRIBUTION OF DIFFERENTIAL SEGMENTS IN *Trillium erectum* L.—*Concluded*

Segments	Chromosome	Material										
		72-D_2-96	72-F_2-96	72-*M*-96	72-*O*-90	72-*P*-96	72-*Q*-96	72-*R*-96	Subtotal	72-*R*-336	72-*R*-676	Total
2 Interstitial	*A*	11	17	15	8	51	14	8	124	20	10	154
	B	0	0	0	5	0	0	0	5	0	1	6
	C	0	0	0	1	0	0	0	1	0	0	1
	D	1	1	0	6	9	0	1	18	0	0	18
	E	19	42	39	30	52	20	13	215	28	19	262
3 Interstitial	*A*	4	0	1	6	2	3	8	24	6	2	32
	B	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
	E	7	1	1	5	20	0	6	40	9	7	56
4 Interstitial	*A*	0	0	0	3	0	1	0	4	2	0	6
	B	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
	E	1	0	0	1	5	0	0	7	2	0	9
Chromosomes affected, %		93	92	96	90	95	96	98		99.7	97	

OTHER CATEGORIES FOUND

1 Terminal + 4 interstitial											1*D*	
2 Terminal								1*D*		1*D*		
2 Terminal + 1 interstitial						3*D*		1*B*		1*D*		
2 Terminal + 2 interstitial								1*D*		1*D*		
5 Interstitial										1*A*		

to say whether or not these differ from each other in the ease with which they may be affected. A classification as to the ease with which segments may become differentially reactive is not without exceptions, chromosomes having sometimes been found in which only the ordinarily less susceptible regions were affected.

Regions, ordinarily unaffected, lying between those that are usually susceptible may show differential reactivity on occasion. In the *A* chromosome of *T. erectum*, for instance, all five proximal segments may become merged to form one differential segment involving about a third of the long arm. Usually in such a case the reaction is less marked in the intervening portions than in the characteristically differential segments. Similarly, in the *E* chromosome of *T. erectum* the whole attachment region between the "normal" differential segments may lose its staining capacity; likewise the terminal segment of the *B* chromosome and the adjacent interstitial segment may merge, often reducing the intervening region to the point where it is morphologically indistinguishable from them. These intersegmental regions may belong to the less susceptible category of differential segments and, therefore, be capable of being affected regardless of the neighbouring regions or they may be secondarily affected by extension of regions already affected. However, since they are usually less markedly affected, the latter interpretation is favoured. It is also possible then that different sizes of both terminal and interstitial regions are due to invasion of normally unaffected areas. This is particularly probable in the interstitials of *T. grandiflorum* where the segments may involve from one to five gyres of the somatic coil and where the diameter and pitch are apparently without much variation. There is no evidence suggesting that stretching of the regions has taken place.

The Rate of Differentiation

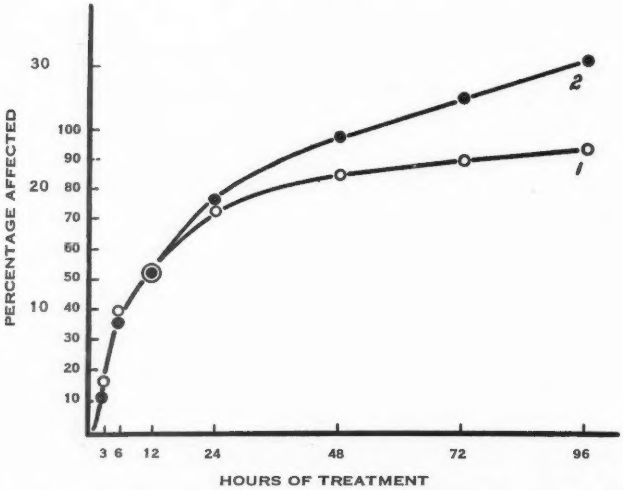
The present investigation has been particularly concentrated on obtaining some knowledge of the rate with which differential segments appear. For this purpose plants of *T. erectum* were exposed to a temperature of 3° C. for times ranging from 15 min. to four weeks. Differential regions first appear after 30 min., and by 96 hr. nearly all chromosomes are affected. Owing to the small number of suitable root tips on the rhizome it was necessary to construct the general curve from a number of overlapping partial curves. Differences between plants are relatively small and may, for the present, be ignored.

Of several ways in which the relationship between duration of treatment and the degree of effect may be determined only two are suitable at present. These are (1) plotting the percentage of chromosomes affected against the duration of treatment and (2) using the regions indicated in Text-fig. 1 as a standard, and plotting the percentage of those obtained against the duration of treatment (Table II). In Text-fig. 2 both curves have been plotted.

TABLE II

DEGREE OF DIFFERENTIAL REACTIVITY IN *Trillium erectum* L. AFTER VARIOUS TIMES OF EXPOSURE TO 3° C.

Plant	Treatment, hr.																													
	1/4						1/2						1						3						6					
	Reactivity																													
	Total chromosomes	Chromosomes affected	%	Possible regions	Regions found	%	Total chromosomes	Chromosomes affected	%	Possible regions	Regions found	%	Total chromosomes	Chromosomes affected	%	Possible regions	Regions found	%	Total chromosomes	Chromosomes affected	%	Possible regions	Regions found	%	Total chromosomes	Chromosomes affected	%	Possible regions	Regions found	%
D_2																									106	51	48	509	79	15
E_2																									77	34	44	314	36	11
F_2																														
M																			295	41	15	1416	44	3	201	67	33	962	70	7
O																									106	38	36	513	41	8
P	103	0	0	495	0	0	109	2	2	525	2	0.4	101	7	7	485	7	1.4	200	29	14	957	30	3						
Total	103	0	0	495	0	0	109	2	2	525	2	0.4	101	7	7	485	7	1.4	495	70	14	2373	77	3	490	190	39	2298	226	10
	12						24						48						72						96					
D_2	173	84	48	826	108	13	172	126	72	823	204	25	103	86	84	502	127	25	127	109	86	619	169	27	142	132	93	681	216	32
E_2	77	37	48	372	46	12	229	162	71	1088	234	22							68	54	85	328	85	26						
F_2	68	31	46	321	34	11																			237	219	92	1151	331	29
M	103	54	52	612	61	10	186	121	65	887	147	17	118	94	80	578	118	20	259	230	89	1237	308	25	238	226	96	1143	315	28
O	85	48	56	410	55	13	194	143	74	918	171	19																		
P																									498	470	95	2389	770	32
Q	79	42	53	496	82	17	101	69	68	491	82	17							164	147	89	786	236	30	113	109	96	532	167	31
R																									104	102	98	500	196	39
Total	585	296	51	3037	386	13	882	621	71	4407	838	19	221	180	81	1080	245	24	618	540	87	2970	798	27	1332	258	94	6396	1995	30
	336						676																							
R	175	174	99	844	337	40	113	109	97	553	210	38																		
Total	175	174	99	844	337	40	113	109	97	553	210	38																		



TEXT-FIG. 2. *Curves showing the rate of differentiation. Curve 1 was obtained by plotting the percentage of chromosomes affected (figures in small type) against duration of treatment and Curve 2 by plotting the percentage of "standard" regions obtained (figures in large type) against duration of treatment.*

These curves cover only the first 96 hr. Owing chiefly to the deleterious effect of long exposure to cold on the root tips only this part of the curve has been investigated in detail.

Curve 1 represents the rate with which the chromosomes become differentially reactive while Curve 2 indicates not only the rate with which the chromosomes are becoming differentially reactive but also the degree to which they are affected. Curve 1 will, of course, reach a plateau when all the chromosomes are affected and this condition is virtually attained after 96-hr. treatment. Curve 2 is limited only by the maximum number of regions that the chromosomes may have, but in so far as present observations go there is little rise in it after 96 hr., when only about 30% of the "standard" regions are affected. This does not necessarily mean that a maximum has been reached but may mean only that the treatment is inadequate. It is also possible that any degree of effect beyond a certain threshold is cell-lethal and this threshold would then be the maximum. The two curves are similar for the first 24 hr. and they then diverge, indicating not only that more chromosomes are becoming differentially reactive but also that the degree to which the chromosomes are affected is increasing. While the curves have not been investigated beyond 96 hr. in sufficient detail to warrant very definite conclusions it is quite obvious that the increase in degree of effect beyond that time is very slight (Table II).

These curves are, of course, compounds of those for the five chromosomes. At this stage of the investigation the authors do not feel that the amount of data available is sufficient to warrant detailed discussion of the individual chromosomes. The percentage of "standard" regions affected after various exposures for each of the five chromosomes has, however, been determined (Table III*a*). From the results tabulated (Table III*b*) it seems that there are quite marked differences between the chromosomes as to the rate with which they show differential segments.

Recovery

Of equal importance to the rate of appearance of the differential segments is the rate with which they disappear on cessation of treatment, but lack of suitable material has made it impossible so far to determine recovery rates in adequate detail. From such experiments as have been performed, however, one definite fact has been determined; namely, that after a 90-hr. exposure to cold the chromosomes return to normal in about 10 hr. at room temperature, most of the recovery occurring within four or five hours. This rapid recovery relative to the rate at which the chromosomes become affected is not unexpected since the rate of mitosis must also be increased at room temperature.

Heterogeneous Chromosome Pairs

Darlington and La Cour (3) have illustrated a number of homologous pairs of chromosomes that differ in number, position, or size of the differential segments or in various combinations of these. They have designated such pairs as "hybrid." The present authors do not feel that this term is justified since it implies genetic hybridity, and prefer, pending further investigation, to call them heterogeneous chromosome pairs.

For the purposes of discussion such pairs have been divided into three classes: (1) pairs in which only one member contains differential segments, (2) pairs differing in the number of these regions, and (3) pairs differing in the length of the terminal regions.

From the fact that increased time of treatment results in a higher percentage of the chromosomes being affected, it seems obvious that Class 1 will eventually be eliminated. Therefore, pairs in which only one member is affected need not be considered further. Within the range of times studied the frequency of heterogeneous pairs of Types 2 and 3 remains relatively constant. These may, therefore, be considered as possible structurally and genetically different or "hybrid" pairs but there are several facts that indicate that such an assumption is not entirely justified. In the first place until the degree of differential reactivity reaches a point of stability a heterogeneous pair must be considered as being potentially similar. In the second place, even if a point of apparent stability is reached (as it seems to be after 96-hr. treatment), it is not known that this stability continues indefinitely. In the third place, even if it does, it may not represent the highest degree to which the chromosomes are capable of being affected; under different treatment it may be higher. Fourthly, the distribution of the affected regions may be subject to chance variation. In the fifth place, heterogeneous pairs do not necessarily indicate genetic hybridity within a plant since a chromosome may fall into

TABLE III*a*

THE DEGREE OF DIFFERENTIAL REACTIVITY IN THE FIVE CHROMOSOMES OF *T. erectum* L.

Chromosome	Treatment, hr.																							
	3				6				12				24				72				96			
	Reactivity																							
	Number chromosomes	Possible regions	Regions found	%	Number chromosomes	Possible regions	Regions found	%	Number chromosomes	Possible regions	Regions found	%	Number chromosomes	Possible regions	Regions found	%	Number chromosomes	Possible regions	Regions found	%	Number chromosomes	Possible regions	Regions found	%
A	99	495	7	1.4	86	430	22	5.1	108	540	43	8.0	166	830	148	17.8	127	635	152	24.0	260	1300	395	30.4
B	99	495	24	4.8	81	405	39	9.6	107	535	78	14.6	179	895	206	23.0	115	575	169	29.4	261	1305	401	30.8
C	98	294	12	4.1	84	252	40	15.7	105	315	59	18.7	177	531	110	20.7	114	342	99	29.0	259	777	254	32.7
D	99	594	11	1.9	89	528	30	5.7	118	708	71	10.0	171	1026	172	16.8	123	738	138	18.7	269	1614	399	24.7
E	99	495	20	4.0	91	455	48	10.5	116	690	92	13.4	186	930	202	21.7	136	680	221	32.4	280	1400	546	39.0

TABLE III*b*

PERCENTAGE OF "STANDARD" REGIONS AFFECTED

Chromosome	Treatment, hr.					
	3	6	12	24	72	96
A	1.4	5.1	8.0	17.8	24.0	30.4
B	4.8	9.6	14.6	23.0	29.4	30.8
C	4.1	15.7	18.7	20.7	29.0	32.7
D	1.9	5.7	10.0	16.8	18.7	24.7
E	4.0	10.5	13.4	21.7	32.4	39.0

more than three categories regarding the number and distribution of its differential segments.

Variability

Differential segments were found to vary greatly in length, this variability persisting throughout the range of treatments studied. Terminal regions were found to be more variable in *T. erectum* and interstitial regions in *T. grandiflorum*. The cause of this variability is unknown. It seems unlikely that it is due to stretching since long regions are not usually narrower than short ones nor is the pitch of the somatic coil noticeably different.

Darlington and La Cour (3) found similar variability in the length of differential segments. They have explained this variability in terminal segments as being the result of "sticking" and failure of normal separation at anaphase. Breaking of the bridge produced by sticking in these regions may result in a duplication in one daughter chromosome and a deficiency in the other. It is difficult, however, to see how such a mechanism could produce variations in the lengths of interstitial regions. In *T. grandiflorum* and *T. stylosum* Nutt. Darlington and La Cour found that 58 out of 127 anaphase figures showed faulty separation of one or more chromosomes. The *T. grandiflorum* material used in the present study has not been examined sufficiently to determine the frequency of faulty separation but out of 13 clear and undamaged anaphase cells only one showed "sticking". Similarly, in 65 cells of *T. erectum* only one case of "sticking" has been found; this was in a cell in which the chromosomes showed no differential segments. It seems unlikely, therefore, that sticking occurs with a high enough frequency to explain the length differences found in terminal regions in our experiments. A more probable explanation of this variation in length appears to us to be extension of the effect to parts of the chromosome adjacent to the differential segment.

Discussion

Darlington and La Cour's suggestion that this phenomenon probably involves the nucleic acid metabolism of the chromosomes is a plausible one, but until more is known concerning nucleic acids in the cell this relationship is purely speculative. They state that the differential segments represent the heterochromatic regions of the chromosomes and identify these with the "chromocentres" of the resting nucleus. While such a relationship may exist our preliminary investigations indicate that far more intensive study must be made before this can be considered as more than an interesting speculation.

Within the differential regions the major effect appears to be in the matrix in that its staining capacity is more reduced than that of the chromonema. The specificity as to position, however, shows that the phenomenon is under the control of the chromonema or the gene-string.

The authors cannot agree with Darlington and La Cour that differences in size, number, or position of differential segments between homologous chromosomes is proof of hybridity whether comparisons are made within or between plants, since heterogeneous chromosome pairs may be equally well considered as potentially similar pairs.

Any interpretation of the shape of the reaction curves presented must await the elucidation of factors that are at present unknown. One of these is probably the rate of mitosis under different temperature conditions.

It appears that not all potentially differential segments are equally susceptible to cold, since certain ones become differentiated far more frequently than others, whereas some occur very rarely even though they appear in a number of different plants. The order in which the regions become affected is not, however, always the same for in rare cases the chromosomes may show one of the least susceptible regions without also having the more susceptible ones.

The fact that the *Trillium* species investigated have different differential patterns suggests that this phenomenon may be of unique value in cytosystematic studies. Such studies are now being made of the genus *Trillium* in this department as a concurrent problem in collaboration with Dr. Pierre Dansereau of the Montreal Botanic Gardens.

In addition to its own obvious utility and interest the existence of differential reactivity is of far wider import in that it is further indication that the chromonema is not uniform throughout its length but is differentiated longitudinally as to chemical structure and behaviour. An explanation of the phenomenon of differential reactivity will, therefore, probably involve a solution to some of the problems of submicroscopic structure. It is the authors' opinion that the eventual solution will be found only by combining the results of experiments undertaken from several points of view, i.e., physical, chemical, and biological.

Acknowledgments

The authors wish to take this opportunity of thanking Professor C. L. Huskins for his interest in the investigation and his helpful suggestions and criticisms. They wish to thank also Mr. E. A. Lods of Macdonald College who supplied seed of the two varieties of rye used. The junior author wishes to acknowledge the financial aid of the National Research Council of Canada whose award of a studentship has made his participation in this work possible.

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THE EXPERIMENTAL INDUCTION OF PARTHENOCARPIC STRAWBERRIES[1]

By A. W. S. Hunter[2]

Abstract

The parthenocarpic development of strawberry fruits was induced by spraying unpollinated blossoms with solutions of indolylbutyric acid, 1-naphthylacetic acid, and colchicine, and by dusting the blossoms with powdered acenaphthene. Fruits also developed from blossoms that had not been directly treated. This is explained on the basis of translocation of the chemical, or some other substance, from treated to untreated blossoms.

It is suggested that colchicine, and possibly the phytohormones, induce parthenocarpy by acting as mobilizers of another substance or substances that move into the ovary and there initiate development.

The experimental production of diploid parthenogenesis in the strawberry as a means of securing homozygosity has engaged the attention of the writer. In an experiment with growth promoting substances and polyploidizing agents the results from the standpoint of parthenogenesis were practically negative, but parthenocarpic fruits were produced in abundance. This account has been prepared since it is believed to add to the knowledge of the methods by which parthenocarpy may be produced.

Methods

During the winter of 1938-1939 plants of three pistillate strawberry varieties, Louise, Portia, and Simcoe, were grown in pots at one end of a greenhouse under conditions such that the chances of accidental pollination were negligible. The blossoms on these plants were treated with the growth promoting substances, indolylbutyric acid and 1-naphthylacetic acid, and with the polyploidizing agents, colchicine and acenaphthene. The indolylbutyric acid, naphthylacetic acid, and colchicine were applied in concentrations of 1.0, 0.5, and 0.25%. A single concentration of one chemical was applied to a separate plant of each variety.

The indolylbutyric and naphthylacetic acids were dissolved in 95% alcohol and the solutions of colchicine were made up in a lanolin emulsion (14). The solutions were applied with an atomizer shortly after the blossom opened. Since acenaphthene is practically insoluble in both water and alcohol, finely powdered crystals were dusted full strength over the stigmas with a camel-hair brush. Treated flowers were tagged and were not sprayed or dusted again unless some of the stigmas had not turned brown after the first application. Since in the majority of cases flowers were deliberately treated once only, the results may be considered as produced by single applications.

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Check treatments were made with 95% alcohol and with lanolin emulsion. Unfortunately, plants of Louise, Portia, or Simcoe were not available for this purpose. Two other pistillate varieties were used instead and will be referred to as Seedling *A* and Seedling *B*.

Results and Discussion

Parthenocarpic fruits have been produced in a number of different genera and species by application to the stigmas of pollen extracts and growth promoting substances and related chemicals (2, 4, 5, 6, 8, 13, 16). The only previously reported work of this nature on strawberries, as far as the author knows, is by Gardner and Marth (2) who produced ripe fruits by spraying the blossoms with 0.1 and 0.05% indolylacetic acid.

The number of flowers treated and the number of fruits that matured from these flowers are given in detail in Table I. Several of the plants failed to blossom and consequently the treatments intended for these plants had to be omitted.

TABLE I

BLOSSOMS TREATED AND MATURE FRUITS HARVESTED

Treatment	Variety									
	Louise		Portia		Simcoe		Seedling *A*		Seedling *B*	
	Number of flowers treated and fruits developed therefrom									
	Tr.	Dev.	Tr.	Dev.	Tr.	Dev.	Tr.	Dev.	Tr.	Dev.
Indolylbutyric acid										
1.0%	4	2	6	6	6	6				
0.5%	8	8	7	7	3	3				
0.25%	8	8	6	6	3	3				
1-Naphthylacetic acid										
1.0%	3	1	5	1	20	2				
0.5%	3	3	1	1	0	1				
0.25%	5	5	8	7	10	9				
Colchicine										
1.0%	9	0	6	1	33	0				
0.5%	0	—	8	7	0	—				
0.25%	14	11	7	7	4	3				
Acenaphthene (dust)	9	3	7	0	12	3				
Check										
95% alcohol							12	0	6	0
Lanolin emulsion							13	0	4	0

No parthenocarpic fruits were produced by treatment of the blossoms of Seedlings *A* and *B* with 95% alcohol or lanolin emulsion alone. The blossoms withered and turned brown after a time in the same way as untreated flowers that had not been pollinated. Genetic differences naturally exist between these two varieties and Louise, Portia, and Simcoe but it is considered unlikely that they are sufficient to invalidate the use of Seedlings *A* and *B* as the checks. Therefore, the 95% alcohol and the lanolin emulsion are believed to have had no part in the production of parthenocarpic fruits.

Parthenocarpic fruits were produced in abundance by all concentrations of indolylbutyric and by the 0.5 and 0.25% concentrations of naphthylacetic acid and colchicine. The 1% naphthylacetic acid initiated fruit development but it also caused considerable damage to the leaves and pedicels. The pedicel injury was so severe that most of the fruits did not reach maturity. The 1% colchicine caused no damage to the plants but only one mature fruit (on Portia) was produced. This was small and misshapen. Under the conditions of their experiment, Gardner and Marth found never more than one fruit on an inflorescence developing to maturity. No such limitation occurred in the present experiment, several well developed fruits being borne on many of the inflorescences following treatments with indolylbutyric acid, naphthylacetic acid, and colchicine. Acenaphthene was not as effective as the other substances in inducing parthenocarpy, although almost all the flowers treated showed some initial fruit development and many of the fruits grew to a length of approximately one centimetre. In all three varieties the mature parthenocarpic fruits were average in size and normal in appearance (Fig. 1) but the achenes were smaller than those on pollinated fruits.

In addition to the fruits that developed from treated blossoms it was also noticed that, on inflorescences bearing flowers treated with indolylbutyric acid, naphthylacetic acid, and colchicine, several flowers that had not been tagged as having been treated showed some carpel enlargement and eventually mature fruits developed. This might have been due to the accidental spraying of unnoticed open flowers. In order to be certain that this was not the case, 53 unopened flowers were tagged at the completion of the spraying programme. In 30 of these flowers there was no development at all but from 12, distributed at random over the different varieties and treatments, ripe fruits developed and in 11 there was initial growth but fruits did not mature. Therefore it is certain that this development was not due to the accidental spraying of open flowers. Since no particular care was taken to prevent the sprayed solutions from reaching parts of the plants other than the blossoms, the stimulation that caused these fruits to develop may have been produced in either of two ways:

1. The presence of the chemical on the outside of the folded sepals may have been sufficient to induce the development of fruits.

2. The chemical or some other substance which it mobilized may have been translocated from the treated to the untreated flowers. The second assumption appears to be the more logical and is supported by a later experiment in which a shield of paper was placed around each blossom as it was being sprayed. The solutions reached no part of the plant except the treated flowers, notwithstanding which several untreated blossoms developed into mature fruits as in the original experiment.

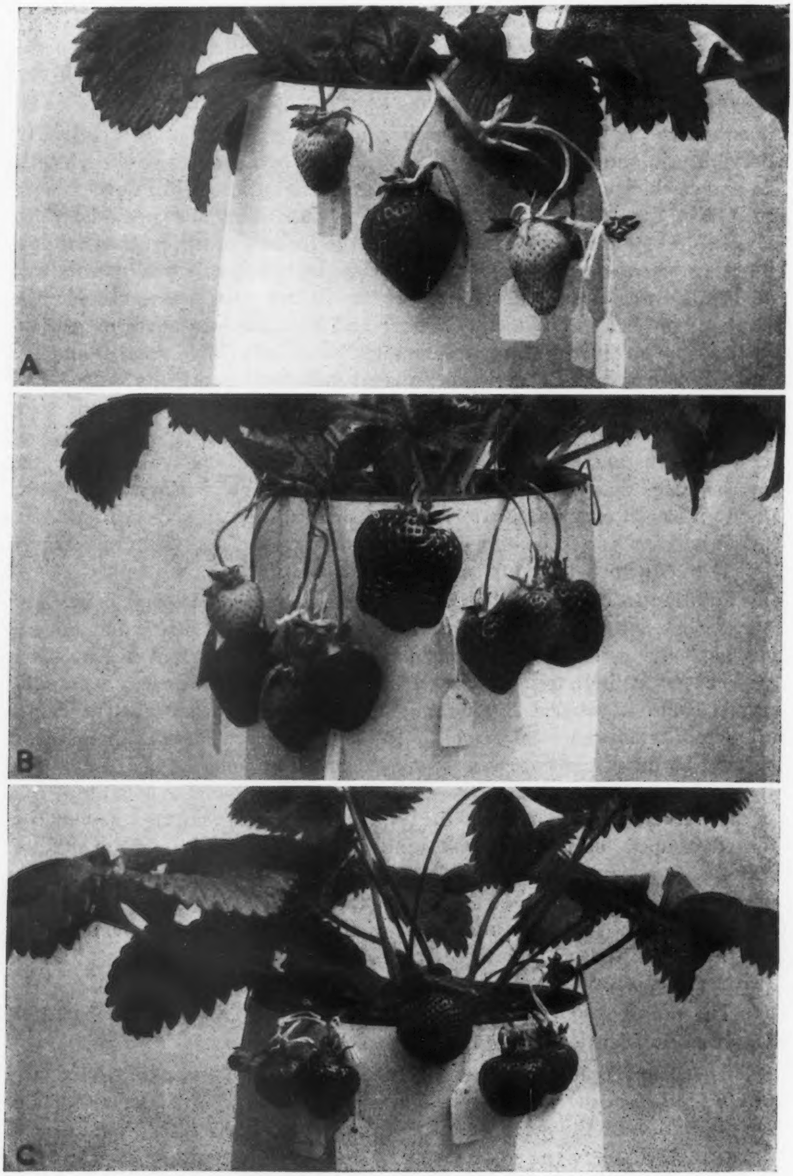

FIG. 1. *A—Simcoe. Blossoms sprayed with 0.5% indolylbutyric acid.*

B—Simcoe. Blossoms sprayed with 0.25% naphthylacetic acid. All the fruits are on two inflorescences.

C—Portia. Blossoms sprayed with 0.5% colchicine. The size of the fruits is a characteristic of the variety.

These fruits which matured were normal in appearance but the achenes were small and underdeveloped as they were in the fruits produced by direct treatment of the blossoms. In the hand pollination of strawberries in the greenhouse the writer has never observed the development of unpollinated blossoms. Presumably the development of untreated flowers is associated with an excess of the stimulating substance in the treated ones. The amount of stimulant produced in pollinated flowers is probably insufficient to affect those not pollinated.

Growth responses other than parthenocarpy were also produced by indolylbutyric acid and to some extent by naphthylacetic acid. With both these substances there was a marked elongation and twisting of the pedicels. Gardner and Marth (2) reported a similar occurrence in strawberry plants sprayed with 0.1% indolylacetic acid. With indolylbutyric acid another effect was the formation of adventitious roots on the scapes in the region of the first node and also at the bases of both scapes and petioles. Bending of the leaves and stems and adventitious root formation have been induced in several plant varieties by local applications of synthetic hormones (10) and by applications to the soil in which the plants were growing (11). In the present experiment no care was taken to protect the foliage or the soil when the blossoms were being sprayed, so that appreciable amounts of the chemical must have been received by the soil and by the leaves and other parts of the plant. This could account for the above results.

The fact that parthenocarpic fruit development in the strawberry may be induced by treatment with colchicine deserves further consideration. Indolylbutyric and naphthylacetic acids both possess growth promoting properties. While little study has been given to colchicine in this respect, not much evidence of growth promoting activity has been found (1, 3), although Havas (9) reported adventitious root formation on the stems of *Impatiens balsamina* following an application of a 2.5% paste of colchicine in lanolin. In the present experiment the plants treated with colchicine exhibited no evidence of epinastic or other growth responses such as were observed in the case of those treated with indolylbutyric acid and naphthylacetic acid. Similar results were also observed in an earlier experiment performed by the writer, wherein various concentrations of indolylbutyric acid, naphthylacetic acid, and colchicine were injected into the trunks of plum trees.

However, more definite information was required on the possible phytohormonal properties of colchicine. In order to obtain such information a series of experiments was conducted with colchicine and indolylacetic acid. Indolylacetic acid was chosen because of its frequent use in phytohormonal studies of this nature. Colchicine and indolylacetic acid were compared for their effect on the curvature of the split stems of etiolated seedlings of *Pisum sativum* (13), the inhibition of lateral bud growth on decapitated tomato plants (10), the production of epinastic responses by tomato plants following applications to the plant (10) and to the soil in which the plant was growing (11). In these experiments the only indication that colchicine has phytohormonal properties was the formation of adventitious root primordia on the stems of tomato plants following local applications in lanolin. Root primordia were produced by the same concentrations of indolylacetic acid but none were found on the check plants. Thus little if any evidence is added to the data already available which would indicate that colchicine possesses to any appreciable extent those properties commonly associated with plant growth substances.

However, since colchicine and the growth promoting substances both initiate parthenocarpy they must have properties in common. It is possible that the induction of parthenocarpy by the growth promoting substances is not altogether due to the fact that they are growth substances, but that it is the effect of one or more of these common properties. Gustafson (7) pointed out that the plant hormones should not necessarily be considered the only factor involved in the initiation of parthenocarpic fruit growth, and suggested, by analogy with Went's (15) terminology, the term "carpocaline" for a substance or substances that, under the influence of auxin, move into the ovary of a flower and there cause growth to take place. According to Went's interpretation, the naturally-occurring auxin in the plant acts as a mobilizer. It would seem possible therefore, that it is this mobilization property which is common to colchicine and the growth substances and which is responsible for the induction of parthenocarpic fruit.

The "seeds" from all the parthenocarpic fruits were sown as soon as the fruits were ripe. One seedling grew from a fruit that developed on the Portia plant treated with 0.25% colchicine. Unfortunately this plant died while still in the seedling stage. The death of the plant was due to an accident and not to any lack of viability. However, root tips had been obtained from which it was determined that this seedling, like Portia, was an octoploid ($2n = 56$). This plant may have been parthenogenetic or it may have been the result of accidental pollination. From the fact that only one plant was secured from so many "seeds", one would suspect the latter, but a selfed population would have been necessary in order to decide this point.

Acknowledgment

The writer wishes to record his indebtedness to Dr. N. H. Grace of the National Research Council, Ottawa, for his helpful criticism of the manuscript.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

I. FACTORS INFLUENCING THE INCEPTION OF DIAPAUSE[1]

BY M. L. PREBBLE[2]

Abstract

This first in a series of five papers includes a review of the literature on diapause and an outline of the life cycle of the European spruce sawfly in Canada, especially the developmental stages within the cocoon. In studies of factors influencing the inception of diapause, evidence has been secured from offspring of stock from one-generation and two-generation areas that there are genetic differences within the species with respect to the capacity for development without diapause. Environmental factors are capable of bringing on diapause, and such factors are obviously operative during the development of the last seasonal generation of "emergent" field populations. However, analysis of weather conditions and incidence of diapause in such field populations failed to indicate correlation between the degree of diapause and any one environmental factor.

Introduction

The European spruce sawfly, *Gilpinia polytoma* (Hartig)[3], discovered in outbreak proportions in the Gaspé Peninsula, Que., in 1930, has since then extended its distribution range in North America to some 150,000 square miles including the Maritime Provinces and parts of Quebec and Ontario in Canada, and the neighbouring states of the United States. Serious mortality of spruce has resulted in the Gaspé Peninsula, and severe defoliation though only moderate tree mortality in other heavily infested areas. Without doubt the insect is the most serious menace to the spruce forests experienced until now in eastern North America.

The problem has been under continual study by the Division of Entomology of the Dominion Department of Agriculture since 1931, and more recently, by the United States Bureau of Entomology and Plant Quarantine, besides

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[3] *The European spruce sawfly in North America, until recently designated Diprion polytomum (Hartig), has been shown by Reeks (59, and a forthcoming paper), Smith (67, 68) and Balch (9), to be distinct from Hartig's polytomum, though identical with another European species not previously distinguished from polytomum. The genus Diprion was revised by Benson (15), a new genus, Gilpinia, being proposed to include several species in the polytomum group. The name Gilpinia polytoma is used in the present paper because a valid name for the recently recognized species is as yet unpublished.*

various state services. The principal features of the bionomics, distribution, and damage have been described in a series of papers by Balch and co-workers (5–9, 11, 12), Atwood (1), Brown (24–27), Brown and Fleming (28), de Gryse and Brown (42), and Gobeil (40, 41), based on work in Canada, and by MacAloney (48, 49), Plumb (56), Baldwin (13), Dowden (36), McIntyre (50), and Peirson and Nash (54), based on work in the United States.

Diapause in *Gilpinia polytoma* has received brief mention in several of the published accounts. Investigations on this phase of the bionomics from 1932 to 1940 leave no doubt that diapause is of fundamental importance in the epidemiology of this insect in North America. Through variations in diapause the species is adapted to climatically different regions where a different number of seasonal generations is produced, and seasonal and regional variations in the degree of diapause are important in determining the nature of the infestations.

This series of five papers describes the results of: (1) studies of genetic and environmental factors in relation to inception of diapause; (2) laboratory and field studies of factors influencing the breaking of diapause; (3) analyses of intracocoon development in different climatic areas, and other bioclimatic relations; (4) studies of the influence of food and diapause upon reproductive capacity; and (5) studies of the role of diapause in epidemiology. There has been no opportunity to carry out purely physiological studies of diapause in the spruce sawfly.

Review of Literature on Diapause

The term "diapause", coined by Wheeler (82) to designate a temporary immobility between the dorsal migration of the embryo and its return to the ventral face of the egg, and extended by Henneguy (43) to include resting periods during any stage in the life cycle, is by Shelford (64) considered to be synonymous with true dormancy, and is defined as "——— a condition in which no further activity or progress can be induced until certain physiological changes of a physico-chemical character have taken place———". The term does not imply any specific causation of the arrest of development, but by definition excludes resting conditions temporarily brought about by subthreshold temperature, etc.

The literature on diapause in insects has increased to such proportions that it is impossible to attempt a complete summary here. Reviews by Shelford (64), Chapman (31), Uvarov (80), Cousin (32), Richards (61), Bodenheimer (16), and Wigglesworth (84) summarize the more important conclusions. The present review is necessarily restricted to brief reference to theories of causation and to the role of various factors associated with the phenomenon.

Sajo in 1896 (cf. 32, p. 301) considered the aestivation of *Entomoscelis adonidis* Palles to be due to narcotization brought on by the accumulation of toxic substances, a view similar to that of Baumberger (14), that diapause is attributable to the obstructive influence of inactive substances accumulated in the cytoplasm after excessive or innutritive feeding, and to Roubaud's (62 and many later papers) hypothesis of auto-intoxication. The latter, in addition to explaining the diapause of the individual, envisages also the accumulation, from one actively developing generation to another, of an "hereditary patrimony of intoxication" which eventually causes the intervention of a diapause period of purification. Bodine (19) proposes a mechanism of diapause in which developmental behaviour is the result of a competition between two opposing factors or groups of factors, diapause intervening when the diapause factor reaches a quantity or potency above a threshold value, development commencing when the diapause factor is no longer able to suppress the developmental factor. Under these various interpretations, the destruction or reduction below a threshold value of the accumulated substances is conceived to be the essential process leading to the resumption of development. According to the hypothesis of Wigglesworth (83), diapause is considered to be due to the temporary failure of the growth promoting factor or hormone (cf. also 61, 84) though the failure might in turn be governed by a diapause factor.

Roubaud's hypothesis has been criticized from several angles. Parker and Thompson (53) failed to find distinct differences in the Malpighian tubules and fat body of diapause and non-diapause larvae of the corn borer. Fink (38) found the assumption of an excretory function of the fat cells to be unjustified, the urates and other products in the cells necessarily signifying nothing more than active metabolism within the fat cells. This, however, is a criticism of detail rather than of principle. Dawson (33) questions the assumption that a somatic acquirement (surcharge of excretory products) can pass on an increasing physiological handicap from generation to generation. Cousin (32), working with several muscid flies upon which the hypothesis was based, failed to find any evidence that diapause in these species is either obligatory, rhythmic, or inherent; on the contrary, it is entirely the result of suboptimal conditions during the life cycle of the individual affected by diapause.

The time at which diapause intervenes in the life cycle of a species is as a rule rigidly fixed. An outstanding example is the *Melanoplus* egg, in which diapause occurs at a definite morphological stage unless forestalled by appropriate treatment (66). A few exceptions include *Reduvius personatus* Linn. which may go into diapause in successive or alternate nymphal instars (58), *Popillia japonica* Newm., which may go into diapause in any one of the three larval instars, depending on the food and temperature (47), and the locust, *Acrydium*, in which nymphs in a growth diapause may occur simultaneously with adults in reproductive diapause (63).

The physiological state (accumulation of toxins, increase of the diapause factor, or inhibition of the growth factor) leading to diapause may be entirely under environmental control, may be genetically determined, or again may vary according to interaction of environmental and hereditary factors, and it is obvious that no one explanation will apply to all species, nor necessarily to different members of the same species. A few examples will indicate the possible variations.

Considering results with *Lucilia sericata* Meig., other Diptera, and the chalcid *Mormoniella vitripennis* Walk., Cousin (32) believes diapause to be the result solely of some unfavourable environmental factor, in the absence of which continuous development is secured. This view, propounded for species with constantly available food, short life cycle, and several to many overlapping generations, is qualified for naturally univoltine species. While on the whole discrediting the role of heredity in diapause, Cousin concedes that it would be extraordinary if univoltine species, accustomed to a fixed climatic rhythm, should yield at once to constant optimal conditions, though she expects that they would do so within a few generations.

Ditman *et al.* (34) concluded that the pupal diapause of *Heliothis armigera* Hbn. is due solely to low temperature during the period of larval development. Diapause in the larvae of *Loxostege sticticalis* (Linn.) is associated with unsatisfactory nutrition and low temperature during development (73), whereas reduced moisture content of the environment and of the host also lead to diapause (74).

Squire (70–72) shows the larval diapause in *Platyedra gossypiella* Saund. to be closely related to changes in the host, and only incidentally to climatic changes. Squire suggests that the remote causes of diapause resolve into a question of unfavourable free water balance, and that the ability of *P. gossypiella* to go into diapause is the result of an evolutionary process enabling the insect to maintain itself on cultivated species of cotton, which, unlike the wild perennial flowering species, have extended periods of dormancy.

The environmental factors leading to diapause may, however, be very obscure. Dawson (33) concluded that the pupal diapause in *Telea polyphemus* (Cramer) is not intrinsically related to nutrition, nor to the rate of larval development as governed either by feeding conditions or by temperature, nor to temperature conditions during the pupal period itself; the important factor appears to be the thermal decline during fifth instar development. This hypothesis, satisfactory for the naturally univoltine stock of Minnesota, is untenable for the bivoltine stock of Nebraska, indicating that the stocks are genetically dissimilar in their reactions to ecological factors.

The phenomenon of widely separated cause and effect may lead to confusion in distinguishing between diapause under control of environment and heredity. For example, incubation of bivoltine eggs of *Bombyx mori* Linn. at 80° F. or higher causes eggs of the subsequent generation to be univoltine, whereas incubation at 65° F. or lower causes no change in the voltinism (77, 79). Dawson (33) produced evidence that incubation of polyphemus eggs at 86° F. either induces pupal dormancy or increases sensitivity of the last larval instar to declining temperature, with the same ultimate effect. Similarly, exposure of very young *Loxostege* larvae to low temperature induces diapause in the full grown larvae even when maturity is realized at 90° F., diapause being entirely absent in larvae reared continuously at this high temperature (73).

In spite of this feature of delayed action of an environmental factor, there are instances where diapause cannot be attributed to environment. The studies of Theodor (75) on *Phlebotomus papatasii* Scopoli are of particular interest because of the borderline between inherent and environmental factors. The fourth instar larvae in field populations go into diapause at the onset of winter rains and at a temperature drop to about 60° F. In insects reared in the laboratory under controlled conditions of temperature, moisture, and food, some of the larvae in occasional broods go into diapause for no apparent environmental cause, although it is known that a lack of, or changed composition of the food, over or under population, and lower temperature are all capable of bringing on diapause. The following facts, however, cannot be explained on the basis of environment: (1) the recurrence of small proportions of diapause larvae in most actively developing broods; (2) the gradual rise in numbers of diapause larvae, up to 90%, in the fall and first winter generation in uniform optimal conditions; (3) the appreciably increased duration of development at this same time; (4) the greater resistance of diapause larvae at this time to efforts to reactivate development; and (5) the return to normal brood development during late winter and spring. To Theodor, these results suggest the existence of a latent inclination to diapause and the influence of persistent cyclical factors. Wigglesworth (84, p. 68) cites Gierke on a similar lengthening of the duration of development of *Ephestia kuhniella* at constant temperatures during the winter months.

The persistence of the rhythm of development in bivoltine stock of the Chinese oak silkworm, *Antheraea pernyi* Guér., transferred from southern Crimea to the less favourable climate of the central area of the Russian Union, is reported by Karlash (44) and Zolotarev (85). Second generation larvae arising from broods started in May were killed by unfavourable conditions in the autumn; but when seasonal development was artificially retarded by cold storage of overwintering pupae until mid-June, first generation pupae were formed in the cool weather of early autumn and went into diapause exactly similar to that of second generation pupae in Crimea. Owing to the failure of climatic impresses during normal first generation development in the central area of Russia to overcome the inherent emergent tendency of the stock, it appears that the culture of the species in this area would depend upon artificial manipulation of the time of seasonal development.

The full grown larvae of the European corn borer may proceed with pupal development at once, or they may go into diapause for several months. Although Kozhanchikov (46) reports that diapause in Russian stocks of this insect is apparently due entirely to temperature conditions during larval development (since there is no evidence of hereditary differences in developmental behaviour of stocks from one-generation and two-generation areas in Russia) there is good evidence that these findings do not apply to American stocks of the insect. Babcock (2) shows that diapause is not exclusively due to environmental factors, since univoltine and bivoltine American stocks tend to persist in their respective types of development when transferred to areas occupied by stocks of the opposite type. Also, diapause (second generation) larvae of bivoltine stock respond with low mortality to developmental conditions after a rest period lasting to November, whereas diapause larvae of the univoltine stock respond equally well only after a rest period lasting until March. Vance (81) reports additional evidence of physiologically distinct strains of the insect in America.

Babcock (3, 4) found that unusually dry conditions during diapause delayed spring emergence and reduced the proportion of bivoltine stock that developed beyond the first generation. The trend towards univoltinism was still greater after a second winter of dry conditions, leading Babcock to conclude that "once the seasonal rhythm has been changed by the continued impress of a certain type of climate, and has been maintained for generations, the reversal to the original type will be much more difficult than at the beginning of the process". The data, which pertain to the inception of univoltinism in a bivoltine stock, and not the converse, seem to the writer insufficient to warrant this conclusion. In fact, the delayed spring emergence may have retarded subsequent development to a time at which seasonal changes, per se, induced diapause, quite independently of any alleged physiological reconstruction of the organism as a result of its previous hibernation experience. The increase in the incidence of diapause in many species with advance of the season (21, 64, pp. 156–157, 70–72) presupposes no dependence on effects produced in a previous generation.

Diapause is more particularly under control of genetic factors in the silkworm, *Bombyx mori*, univoltine races of which have a prolonged embryonic diapause in each generation; other races produce one or more generations completely free of diapause. In crosses, univoltinism tends to be dominant (30, pp. 210–216) but segregation is not entirely clear-cut because of the influence of the somatic cells upon the developing germ cells, the resulting eggs having characteristics of the mother's race (77, 79). A similar result is obtained when ovaries are grafted into female larvae of a different racial stock (cf. 84, p. 9).

Different hereditary types of pupal development in *Deilephila euphorbiae* Linn., with respect to the pupal period (absence of diapause, or diapause of variable duration), respiratory rate, etc., have been described by Heller (cf. 16, pp. 149–152, 84, p. 68). Goldschmidt reports racial differences in *Lymantria dispar* Linn., where the races are univoltine but genetically distinct in duration of diapause in the egg and in the rate of larval development (cf. 16, pp. 149–152, 35, pp. 156–157).

The possibility of obscuring racial differences with respect to diapause, by crossing and production of heterozygotes, and the domination of genetic factors by prevailing environmental conditions, must not be overlooked. The bimodal distribution, including earlier (emergent) and later (diapause) progeny of single polyphemus moths reared in the laboratory, suggests to Dawson (33) that the Minnesota stock is heterozygous for voltinism. In nature, climate prevents the development of the emergent elements beyond a single generation and thus ensures their survival. In the laboratory the emergent elements continue development without diapause.

Characteristic physiological alterations during diapause include cessation of mitosis (65), and a reduced respiratory metabolism (18, 21, 69). Reduced metabolism is to be regarded as a result of diapause (20) rather than its cause as was suggested by Knoche (45) and Tuleschkov (78). Many other physiological characteristics during diapause have been discussed in recent literature, but are outside the scope of this paper.

The resumption of development of insects in diapause is promoted by a number of factors. The need for rest at low temperature is almost a universal requirement. The temperature may have to be near the freezing point (29), or merely below the threshold of development (2, 39). While diapause is most efficaciously overcome at low temperature, in some instances it may also be overcome gradually at developmental temperature (21, 66). The response to favourable temperature is often more rapid in relation to the duration of exposure at low temperature (17, 21).

The coincidence of resumption of development with moisture changes in the natural environment (23, 57) and the stimulating effect of water addition either during the normal diapause period (2, 3, 4) or at the time of its normal cessation (60, 71, 76) clearly indicate the important role of water in breaking diapause. High humidity is not as effective as contact water in most insect species.

Other stimuli appearing to alter the normal physiological processes and frequently leading to a breaking of diapause, include singeing, friction, wounding, electrical or mechanical shocks, irradiation, treatments with chemicals, etc. (22, 43, 55, 61, 63, 84).

Although the physiology of diapause is far from being understood, the adaptive value of the phenomenon is recognized as leading to a synchronization of the organism with its environment, especially where survival depends upon timely occurrence of a resistant stage. The latter is characteristically the stage affected by diapause (16, pp. 149–152, 84, p. 69). What appears to be an interesting exception occurs in the fall cankerworm, *Alsophila pometaria* Harris, pupae of which, formed in early summer, have a diapause lasting until autumn. Adults emerge and oviposit in November and early December. A considerable proportion of the eggs will hatch without exposure to subthreshold temperature (10, 39), and although the egg is very resistant to low temperature, the winter hardiness of the pupa is apparently unproved. The summer pupal diapause ensures that eggs occur at a season when their untimely hatch cannot take place.

Diapause in Relation to the Life Cycle

The spruce sawfly overwinters as a larva within a cocoon in the forest floor. Seasonal development commences in April to June, according to climate, and adults emerge after several weeks to lay the eggs singly in spruce needles. There are five feeding larval instars, and at the fifth moult there appears a non-feeding instar that evacuates the alimentary tract through the anus, drops to the ground, and spins the cocoon. The cocooned larvae, in areas where there is a single annual generation, go into diapause that lasts until the following spring or for several years. In areas in which there are two or more annual generations, the cocooned larvae of the last seasonal generation go into diapause until the following spring or later, whereas those of the earlier seasonal generation go into diapause or develop at once, according to circumstance.

DEVELOPMENTAL STAGES WITHIN THE COCOON

The last larval instar of *Gilpinia polytoma*, as of numerous other sawfly species, has a number of successive developmental phases frequently but unsatisfactorily included under the term "prepupa". This looseness of definition is confusing in studying insects in which the successive larval phases, though not separated by a moult, exhibit a variety of habits. The spruce sawfly larva appearing after the fifth moult, though non-feeding, is active on the trees for a day or two and reacts similarly to the earlier instars; for convenience, this phase has been called the free-living sixth instar. Beginning with evacuation of the alimentary tract a day or two after the fifth moult, the reactions to light and gravity are reversed, and morphological changes associated with the later phases are initiated. The terms "eonymph" and "pronymph", used for sawflies by Eliescu (37), Nägeli (52), and others, and for *Exenterus abruptorius* (Thunberg) by Morris (51), are adopted here for the distinct phases of the cocooned prepupal larva.

The *eonymph*, or first larval phase within the cocoon (Fig. 1A), is practically filled with fat body in the form of a many-folded, single-layered blanket of large cells held together by connective strands. The ovaries, with ovarioles already quite distinct, measure about 0.55 by 0.25 mm. and lie within the folds of fat body, one on either side of the slender intestinal tract in the hind portion of the fifth abdominal segment. The rare males are identified by the cluster-like testes in the corresponding segment. The abdominal prolegs are withdrawn, and the wing rudiments appear through the integument of fixed material as small white lobes. The eonymphal phase of the prepupal larva is the phase in which diapause characteristically occurs.

The *pronymph* is the phase in which structural reorganization and development of imaginal rudiments begin. The body becomes shorter, thicker, nearly straight, the intersegmental constrictions more prominent, the thoracic segments swollen, and the abdominal prolegs further reduced (Fig. 1B). The earliest pupal structure to become apparent is the eye, which, beginning as a narrow arc under the posterior margin of the larval ocular sclerite (Fig. 1C) gradually enlarges and moves dorso-caudad, attaining its full size and red colour prior to the moult into the pupa (Fig. 1G). The ovaries of the fully developed pronymph are about 1.65 mm. long, with clearly defined oocytes and nurse cell follicles. The larval skin is loose and overlies a copious supply of moulting fluid. The duration of pronymphal development from the first evidence of the pupal eye to ecdysis averages about 17 days at a mean temperature of 49° F.

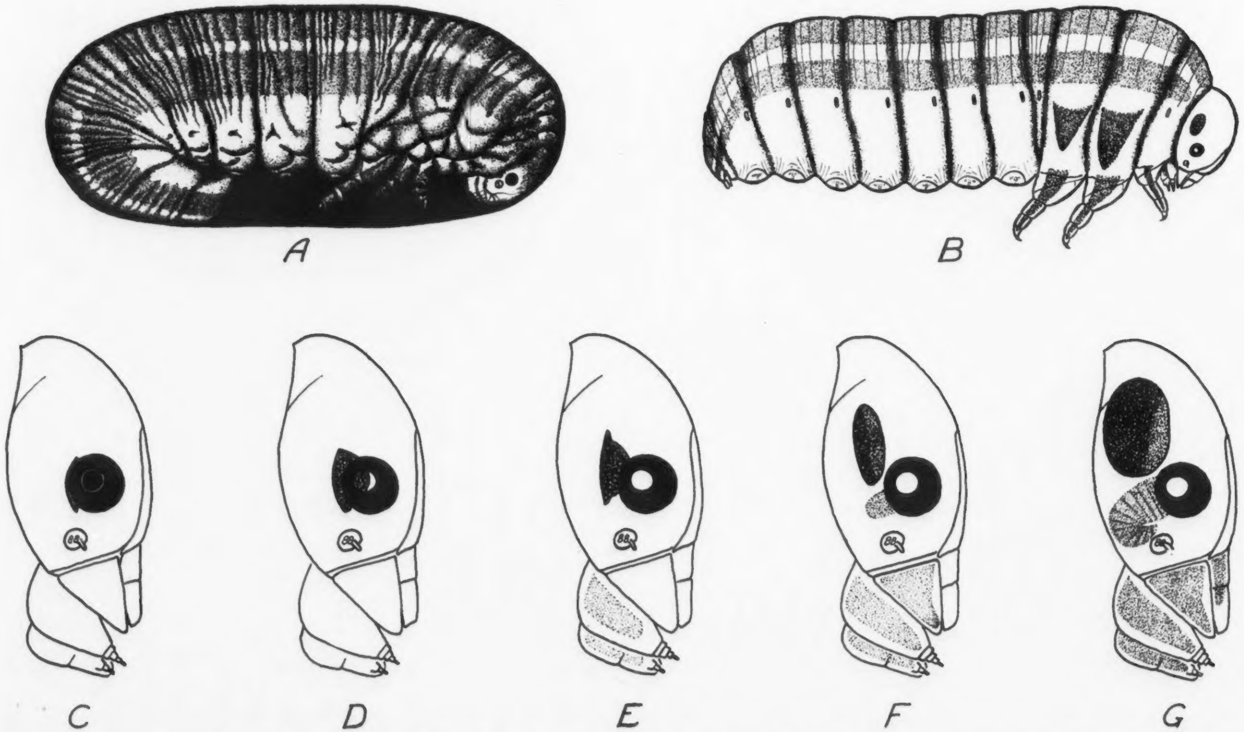


FIG. 1. *Eonymph and pronymph of Gilpinia polytoma. A, eonymph within cocoon; B, advanced pronymph; C to G, head showing successive stages in pronymphal development. (Drawing of eonymph by W. A. Reeks.)*

The first appearance of the pupal eye (Fig. 1C) has been used very extensively in these studies as a criterion of the continuance or resumption of development; its use in this respect is equivalent to that of the male gonads in European corn borer larvae by Parker and Thompson (53).

The pupal stadium averages 11 to 12 days at a mean temperature of 54° F., and 13 to 14 days at 50° F.

The newly transformed adult remains in the cocoon one to several days during which it dries and hardens. By thrusting the left mandible through the cocoon wall, then revolving inside the cocoon to shear off a lid, the adult escapes.

General Characteristics of Diapause

The development of eonymphs in diapause is resumed only after one or several periods of rest at low temperature. The rate of metabolism during diapause is greatly reduced, even at favourable temperature conditions, as demonstrated by preliminary tests of oxygen consumption, by the very slight reduction in dry weight over extended periods, and by the lack of a consistent downward trend in reproductive capacity of females emerging in successive years from a common source.

Pronymphs whose development is curtailed by the onset of winter conditions occasionally occur in a state of true diapause, resuming development only after a period of cold-rest. Pronymphal diapause was detected in overwintering Gaspé populations in 1934 and 1935, when from 2% to 8% of the overwintering individuals in different localities consisted of pronymphs in the early stages of development. Pronymphs in sample lots incubated prior to mid-November remained in diapause indefinitely, whereas there was a prompt resumption of development in sample lots incubated in mid-November and later. More typically, pronymphs occurring in the overwintering populations in Gaspé and in south central New Brunswick have continued their development irrespective of a rest period. Pronymphal diapause may therefore be regarded as unusual, and has never been observed during the summer season.

Hardiness of Eonymphs in Diapause

Eonymphs in diapause are very resistant to low temperature. The first mortality, about 1%, results from exposure of the cocoons to 0° F., and complete mortality results only after exposure to −20° F., when the exposure is gradual, such as experienced during a cold night in winter. Lethal temperatures are never experienced in the moss layer of the natural habitat (9).

Pronymphs are approximately as resistant to intense and prolonged cold as the eonymphs, but pupae and adults are much more susceptible. In a series of cocoon samples containing pupae and unemerged adults, that were held in storage close to 32° F. for several months and then opened for analysis, pupal mortality ranged from 35% to 67%, and adult mortality from 70% to 100%, in different sample lots.

Eonymphs are also more resistant to excessive moisture and to high temperature than are the later developmental stages within the cocoon. Many thousands of cocoons have been subjected to a variety of laboratory and field conditions in a study of the factors influencing resumption of development, providing extensive mortality statistics for each stage. It has generally been noted that the same environmental conditions that promote a high percentage of development also cause an increased mortality*. In the tabulation of representative mortality statistics (Table I), the death rate for each stage is the percentage relation between the number of dead individuals found at analysis of the cocoons, and the total number of individuals that attained, or developed beyond, the stage in question. The calculations therefore provide strictly comparable statistics for the various stages and for different experimental lots.

Mortality generally increased at higher temperature and in more frequent contact with water. Eonymphal mortality was generally the lowest and

TABLE I

MORTALITY STATISTICS FOR THE VARIOUS STAGES WITHIN THE COCOON IN RELATION TO TEMPERATURE AND MOISTURE CONDITIONS DURING THE PERIOD OF DEVELOPMENT

Material	Incubation conditions			No. of cocoons	Total devel., %	Mortality rate, %			
	Temp.	Rel. hum., %	Immersions			Eonymphs	Pro-nymphs	Pupae	Adults
Gaspé cocoons	74° F.	100	1 †	305	75.4	0.7	5.2	15.0	2.9
			3 †	290	69.7	1.7	4.5	11.3	2.4
			7 †	284	85.9	4.2	14.3	25.4	4.0
			14 †	295	87.5	6.4	19.8	33.3	4.7
			21 †	289	82.7	7.3	28.0	36.5	6.5
Gaspé cocoons	55° F.	100	None	144	16.0	1.4	0	0	5.0
			2-day immersion	702	24.1	0.4	1.8	10.1	3.8
	65° F.	100	None	141	19.1	0	3.7	0	0
			2-day immersion	593	28.5	0.5	0.6	3.3	4.1
	74° F.	100	None	445	97.1	2.0	6.7	19.1	9.6
			2-day immersion	970	97.0	2.5	10.2	19.1	6.8
English Settlement, N.B. cocoons									
a. Collected in fall	74° F.	100	None	9743	98.8	1.0	4.8	14.9	12.4
			2-day immersion	9573	98.9	0.8	4.3	15.1	9.1
b. Collected in May	74° F.	100	None	623	98.4	1.0	5.1	18.6	17.9
			2-day immersion	627	99.5	0.3	2.9	21.3	17.7
c. Collected in May, left in natural habitat.									
Protected from rainfall				2598	32.6	1.1	1.2	5.0	7.5
Exposed to rainfall				4675	88.7	1.8	3.1	13.1	18.1

† *Immersions at rate of one daily.*

**For this reason, experiments on the rupture of diapause must not cease with the recording of emerged adults since these represent only a part of the total number of individuals whose diapause was successfully overcome.*

pupal mortality the highest, though the latter was occasionally exceeded by adult mortality. Under natural conditions in the forest, mortality within the cocoon (exclusive of predatism) is generally less than in the incubator, seldom exceeding 1% in eonymphs and pronymphs and 2% to 3% in pupae and unemerged adults, during the summer season. Excessive rainfall significantly increases the mortality, as may be appreciated from the following tabulation based on records in five localities in northern New Brunswick and Gaspé during the very wet season of 1939 (over 20 in. of rainfall in July and August).

Stage	Mortality rate, %	Stage	Mortality rate, %
Eonymph	2.4	Pupa	18.3
Pronymph	4.5	Adult	11.8

The much greater resistance of eonymphs assumes an increased significance in view of the prolonged diapause period frequently experienced in this stage; the normal period of pronymphal and pupal development is two to three weeks, and the newly transformed adults normally remain in the cocoons for only a few days.

Factors Influencing the Inception of Diapause

GENETIC FACTORS

Populations of sawfly lines were extensively reared at Fredericton from 1934 to 1938, to determine the range of variability in developmental behaviour within the species, and to check the possibility of relationship between origin of the stock and its type of development. A brief summary of the conclusions derived from this project has been given by Balch (9), but in view of the importance of the conclusions in relation to the interpretation of regional differences in populations, a more complete account of the results appears to be warranted.

The practice is to place newly emerged females, singly, in small globe cages enclosing a spruce twig in contact with water in the basal portion of the cage. The original twig accommodates the full complement of eggs and serves as food for the young larvae. Beginning about the second instar, the larvae are transferred to fresh foliage at intervals of a few days. At 74° F., cocoons are spun in the foliage or in moss provided for the purpose, beginning about the 28th day. In emergent lines, adults appear from about the 38th day to the 45th day, but in non-emergent or diapause lines development ceases at the eonymphal stage and, if the cocoons are left indefinitely at the incubator temperature, it is not resumed. The cocoons are left in the incubator for at least a month, and are then either opened for analysis or put into storage for several months, after which they are again incubated. The populations have been reared mainly at temperatures between 70° and 74° F. Humidity, although not readily controlled because of the foliage and because of the difficulty of securing circulation within the tulle-covered globes, was generally about 80% or higher.

Offspring of Gaspé Stock

Eighty-five females of Gaspé origin, taken at random from field collections over a period of years, produced families of cocoons in the incubator. Based on the first generation, 70 families with a total progeny of 373 cocoons, were classed as diapause lines since no development beyond the eonymph occurred without a period of cold-rest. The other 15 families were classed as emergent lines, development being uninterrupted in 72% of the 118 cocoons produced.

Second generations were obtained for 10 of the lines classed as emergent in the first generation. In five of the lines there was some emergence from the second generation cocoons. Altogether, 25% of the 398 second generation cocoons of the so-called emergent lines of Gaspé origin continued development without diapause.

The third generation was obtained for three lines that were emergent in the two preceding generations, and for one line that was carried on only by storing cocoons of each preceding generation. Only 5 of the 270 third generation cocoons continued development without diapause, and these all pertained to a line emergent in each preceding generation.

The last-mentioned line was carried to the fourth generation in the incubator, but all of the 27 cocoons produced went into diapause. Repeated attempts to establish a continuously developing line of Gaspé stock were unsuccessful, owing to the complete intervention of diapause in the early generations.

In addition to the populations reared in the incubator, 100 unrelated females of Gaspé origin were reared outside at Fredericton, starting in late May or early June and were therefore concurrent with the first generation of the field population normally developing in the Fredericton district. All 716 cocoons in 92 families went into diapause, and in the other eight families there was a partial emergence, affecting 8 of the 50 cocoons produced. This represents an emergence of about 1% from first generation outside rearings of Gaspé stock in the Fredericton district.

Offspring of New Brunswick Stock

Thirty-five females from different parts of south central New Brunswick produced first generation progeny in the incubator. On the basis of the first generation cocoons, seven families (53 cocoons), in which there was no development beyond the eonymph, were classed as diapause lines. In the other 28 families (296 cocoons), there was an emergence without diapause of 81%, and, in over one-half the families, of 100%. Six other families, having 68% emergence without diapause, started in the insectary and finished in the incubator, were all emergent lines.

Successive generations of nine lines, classed as emergent in the first generation, were reared in the incubator at the rate of about nine generations a year. Usually a few eonymphs of each generation went into diapause, and occasionally all the progeny of one female, but as a rule from two to five families were reared for each generation of each line and the succession of generations was unbroken. Owing to the pressure of other work, several of the lines were discontinued after 6 to 12 generations, but one line was carried to the 22nd and another to the 23rd generation. Finally the stocks were wiped out in 1938 by a virus disease introduced from the field and since then it has been impossible to rear larvae beyond the earlier instars. This circumstance has prevented the testing of new lines from field stock.

The varying percentages of insects in diapause in the different generations of a line remained unexplained, but since the variations were not parallel in the populations reared concurrently they were obviously not due to cyclical or seasonal factors. There was a sufficient variability in the degree of diapause in the different lines, as shown in the accompanying synopsis, to suggest the possibility of inherent differences between lines in the capacity for continuous development.

Line	No. of generations	No. of cocoons	Insects in diapause, %
1B	23	631	29
5	6	169	12
9	6	210	29
14	22	2306	8
21	10	319	11
22	12	495	4
24	12	669	0.6
25	12	882	5
26	9	432	4

Other evidence of inherent differences in the capacity for continuous development was provided by a number of New Brunswick lines which, having a high degree of diapause in the first generation, were reared for two or three further generations to determine whether there might subsequently be a significant departure. The data in the synopsis below indicate that the determination based on the first generation was close to that based on the entire line.

Line	Insects of first generation in diapause, %	Number of generations	Number of cocoons	Average number of insects in diapause, %
21F	27	3	81	49
32A	86	3*	25	96
32D	100	4**	55	89

**All second generation cocoons went into diapause.*

***Second generation reared after storage of first generation cocoons. Fourth generation cocoons went into diapause, and development was resumed only after two periods of cold-rest.*

The data presented in the foregoing sections indicate distinct differences between the Gaspé and south central New Brunswick stocks of *Gilpinia polytoma*. Comparatively large proportions of the New Brunswick population can be established as continuously developing lines in optimal conditions, though a small and variable percentage of the progeny goes into diapause. But there also exist lines that either fail to develop beyond the first generation, or in which the tendency towards diapause is so great that subsequent generations are reared with difficulty. The Gaspé population, on the other hand, appears to be composed predominantly of lines in which diapause intervenes after a single generation, and, to a lesser extent, of partially emergent lines.

Transfers of Stock to New Climatic Areas

Wild stock from south-central New Brunswick and stock from line No. 14 were transferred to the interior of the Gaspé peninsula and reared in the open with check populations of Gaspé stock. The results are summarized in Table II.

TABLE II

PERCENTAGE OF INSECTS IN DIAPAUSE IN POPULATIONS REARED FROM NEW BRUNSWICK AND GASPÉ STOCK IN CENTRAL GASPÉ

Year	Stock	No. of families	No. of cocoons	Insects in diapause, %
1935	Gaspé (wild)	4	74	100
	McNamee, N.B. (wild)	2	46	100
	Nashwaaksis, N.B. (wild)	3	44	100
	N.B. line No. 14	6	59	44
1936	Gaspé (wild)	5	97	100
	N.B. line No. 14	1	19	89
1939	Gaspé (wild)	–	–	100*
	Douglas Harbor, N.B. (wild)	3	27	4

**A strict check on rearing was not obtained in 1939, but there was no emergence from several hundred of the earliest cocoons of the Gaspé field population.*

Gaspé stock reared in these three and in previous years, and newly spun cocoons of the field population of central Gaspé from 1932 to 1939, consistently failed to continue development under Gaspé climatic conditions. Two isolated exceptions occurred out of many thousands of newly spun cocoons that were under observation. Wild stock from McNamee and Nashwaaksis produced only a single generation in central Gaspé, but there was sufficient development in the first generation progeny of stock from Douglas Harbour and line No. 14 to preclude the possibility that the result was due to chance. Clearly the developmental behaviour of these latter stocks under Gaspé climatic conditions was influenced by their genetic constitution.

The second generation rearings of these New Brunswick stocks in Gaspé developed to partly grown larvae that were caught by the onset of winter conditions in early October. It is highly improbable that any part of a second generation could ever survive in central Gaspé.

As for the transfer of Gaspé stock to south central New Brunswick, it has already been noted that only 8% of the "lines" took advantage of the improved climatic conditions to continue development without diapause. This affected approximately 1% of the first generation progeny, under conditions at which a large percentage of New Brunswick stock develops without diapause.

ENVIRONMENTAL FACTORS

Studies of environmental factors in relation to the inception of diapause were made possible by the existence of lines capable of continuous development at optimal conditions. Branches of these lines were reared at variable temperature and on different types of foliage. Field studies were carried out in relation to the inception of diapause in populations in a two-generation area.

Effect of Foliage

The incidence of diapause in seven lines at different seasons of the year is summarized in Table III. The foliage on which these were reared included the needles of all years; white spruce was the characteristic host, though red spruce was used to some extent for line No. 14. The percentage of insects in diapause varied from season to season, but the fluctuations were not consistent in the various lines, therefore providing no evidence of a significant correlation between seasonal effects of the foliage as a whole, and diapause.

The preferred food of the larvae is the older foliage, and forcing them onto the new foliage, especially of black and red spruce, results in slower development, smaller size, and reduced survival. Later in the season the larvae

TABLE III

INCIDENCE OF DIAPAUSE IN SEVEN LINES REARED IN THE INCUBATOR AT DIFFERENT SEASONS

Season	1B		14		21		22		24		25		26	
	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %
Jan. - Feb.	140	2	315	9	98	1	74	0	177	0	157	0	198	1
Mar. - April	15	20	410	10	57	4	131	0	65	0	11	18	40	2
May - June	89	18	202	2	94	21	44	5	89	1	74	16	29	14
July - Aug.	137	34	380	2	18	50	121	1	28	11	37	0	10	30
Sept. - Oct.	143	58	413	13	44	5	98	15	69	0	68	0	29	7
Nov. - Dec.	107	31	586	7	8	25	27	0	241	0	535	6	122	0
Average for line		29		8		11		4		0.6		5		4

occasionally feed on the new foliage and in severely defoliated forests this may be the only alternative to starvation. Accordingly, it is of interest to determine whether there is any relation between the type of food and diapause. Branches of line No. 14 were reared on various types of foliage for several generations while concurrent generations of the main stem of the line were maintained on the "standard host", i.e., old and new foliage of white spruce. The results of the tests are shown in Table IV.

TABLE IV

INSECTS IN DIAPAUSE IN BRANCHES OF LINE No. 14 REARED IN THE INCUBATOR ON VARIOUS TYPES OF FOLIAGE, %

—	No. of generations	No. of cocoons	No. in diapause, %
1. Red spruce (old and new)	5	148	1.3
Standard host	5	341	5.3
2. Black spruce (old and new)	5	92	8.7
Standard host	5	251	5.2
3. White spruce, very rank growth	8	262	5.3
Standard host	8	351	6.3
4. White spruce, old foliage (Oct.–Dec., 1935)	2	23	0.0
Standard host	2	217	2.3
5. White spruce, old foliage (July–Dec., 1936)	4	152	18.4
Standard host	4	292	4.1
6. White spruce, new foliage (Oct., 1935–Jan., 1936)	3	64	1.6
Standard host	3	242	2.1
7A. White spruce, new foliage (July–Sept., 1936)	2	54	72.2
Standard host	2	145	0.7
7B. White spruce, new foliage (Oct.–Nov., 1936)	1	76	43.4
Standard host	1	49	22.5
7C. White spruce, new foliage (Dec., 1936)	1	49	0.0
Standard host	1	98	0.0

NOTE:—*The "standard host" consisted of white spruce twigs with old and new foliage.*

There were obviously no differences in diapause, attributable to the type of foliage, in Tests 1, 2, 3, 4, 6, and 7C. In test No. 5, the significantly higher degree of diapause on the old foliage alone would suggest some causal relationship between the absence of new foliage and inception of diapause. This interpretation cannot be accepted, firstly, because the new foliage is only rarely eaten in the presence of abundant old foliage; and secondly, because the duplicate test, No. 4, failed to provide similar results. The increased diapause in test No. 5 must be attributed to unknown factors, chance alone being a very remote possibility (*P* less than 0.01).

The increased diapause in insects reared on the newly formed needles of white spruce suggests a definite relationship between seasoning of the new foliage and inception of diapause. Thus in the insects reared earliest on the new needles (test No. 7A) there was the highest percentage of diapause; this percentage gradually declined until, in insects reared in late fall and winter (Tests 6 and 7C) both on the current year's foliage and on the standard host, it was identical. The chemical changes associated with ageing of the new needles have not been investigated, and even though the experimental evidence may be taken to support an hypothesis that diapause in emergent stock is associated with changing nutritive conditions of the host, such an hypothesis fails to account for, first, the success of incubator rearings at all times of the year on freshly cut foliage; and second, the virtual insignificance of the less palatable new needles in the diet of most field populations that nevertheless experience a sharp mid-seasonal increase in diapause in south central New Brunswick.

Effect of Temperature

Twenty-four families descended from the same great-grandmother in line No. 14 were reared in the incubator until the larvae were in the second and third instars. Nine families were then transferred to an environment with fluctuating temperature (39° to 71°, mean 54° F.), six families were left in the incubator, and the remaining nine families were placed alternately in the incubator and the lower fluctuating environment until they had experienced 20 daily exposures of 16 hr. each to the fluctuating temperature. At the time of spinning, all cocoons were returned to the incubator. As shown in the accompanying synopsis, there was a clear relation between temperature at which they were reared and incidence of diapause.

Temperature conditions during late larval development	Number of cocoons	Insects in diapause, %
73° F.	88	0
Alternating between 73° and cooler environment	103	31
Cooler environment	135	72

Five families of line No. 14 were started in the field in central Gaspé in August, 1935. From then until October 11 the temperature was low, averaging only 45° F., and had dropped below the freezing point on 18 occasions, the absolute minimum being 20° F. On the latter date, the larvae that were mostly in the third instar, were transferred to the incubator to complete their development. Of 23 cocoons produced, only one went into diapause.

From these two experiments it may be concluded, (1) that fluctuating and very low temperature during the first three instars does not necessarily induce diapause in the eonymphs; and (2) that diapause may be brought into an emergent line by fluctuating and suboptimal temperature during the latter part of larval development. This recalls the conclusion reached by Dawson (33) that the fifth instar of the polyphemus moth (Minnesota stock) is the critical stage at which sensitivity to temperature determines the course of subsequent development. Unfortunately, attempts to localize the suspected critical stage in *Gilpinia polytoma* were fruitless owing to the destruction by disease of about 100 family populations of pure line stock set up for a complete experiment.

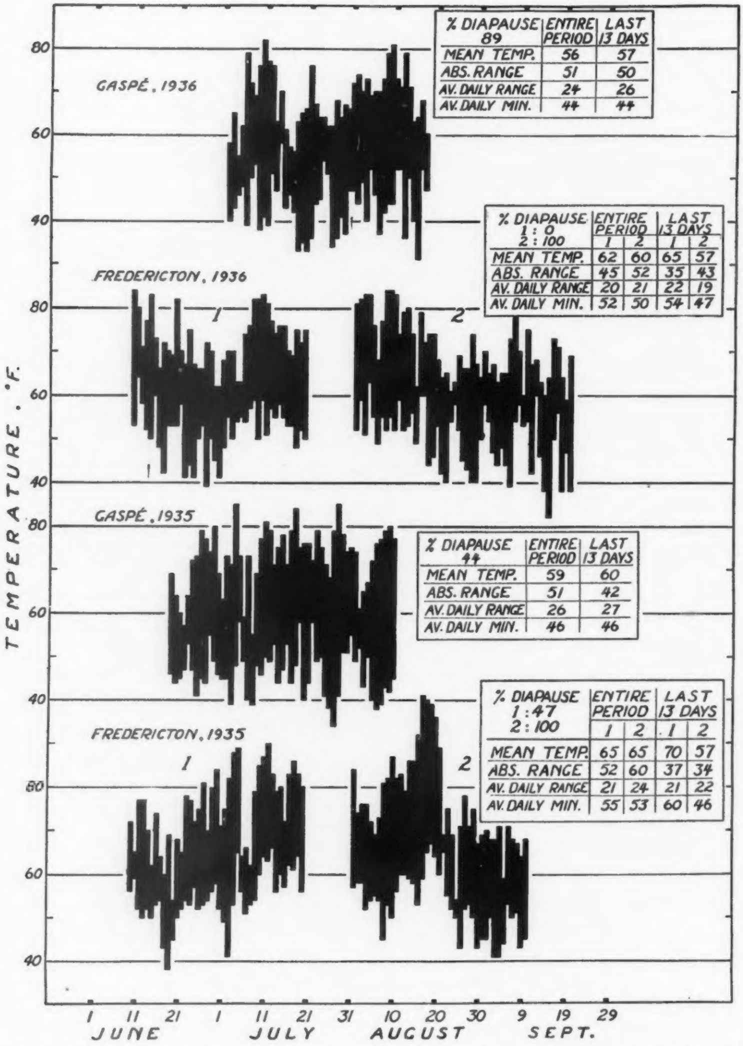
The results described above were checked by rearing concurrently branches of pure lines in the incubator and in the open insectary at Fredericton (Table V). Diapause in the incubator insects was uniformly low, and while variable in the early outside populations (31% to 51% in 1935; 0% in 1936), it was 100% in all late outside populations. The following observations were of particular interest: (1) the 100% value in late outside populations applied equally as well to progeny of females reared in the incubator during the preceding generation, as to progeny of females reared outside in the preceding generation; (2) the diapause induced in the emergent lines was in most cases overcome by a short period of cold-rest, though without this, development was not resumed.

TABLE V

SUMMARY OF THE INCIDENCE OF DIAPAUSE IN THREE PURE LINES REARED OUTSIDE AND IN THE INCUBATOR AT FREDERICTON, N.B.

Line	Year	Time when rearings started	Outside		In incubator	
			No. of cocoons	No. in diapause, %	No. of cocoons	No. in diapause, %
5	1935	Early June	26	46	58	2
		Late July–early August	96	100	—	—
	1936	Early June	19	0	—	—
		Late July–early August	54	100	—	—
9	1935	Early June	13	31	27	11
		Late July–early August	59	100	—	—
14	1935	Early June	55	51	51	2
		Late July–early August	30	100	48	6
	1936	Early June	37	0	118	0
		Late July–early August	135	100	114	1

The temperature fluctuations during larval development in the outside populations of pure line stock at Fredericton and in Gaspé are represented graphically in Fig. 2. Due to the fact that not all larvae within each group developed simultaneously, it has been necessary to chart the fluctuations for the period during which the preponderant number of individuals in each group developed. A summary of the temperature conditions during each period, and during the final 13 days of each period (average fifth stadium), as well as the average percentage of insects in diapause, are shown in the insets



% DIAPAUSE 89	ENTIRE PERIOD	LAST 13 DAYS
MEAN TEMP.	56	57
ABS. RANGE	51	50
AV. DAILY RANGE	24	26
AV. DAILY MIN.	44	44

% DIAPAUSE 1 : 0 2 : 100	ENTIRE PERIOD		LAST 13 DAYS	
	1	2	1	2
MEAN TEMP.	62	60	65	57
ABS. RANGE	45	52	35	43
AV. DAILY RANGE	20	21	22	19
AV. DAILY MIN.	52	50	54	47

% DIAPAUSE 44	ENTIRE PERIOD	LAST 13 DAYS
MEAN TEMP.	59	60
ABS. RANGE	51	42
AV. DAILY RANGE	26	27
AV. DAILY MIN.	46	46

% DIAPAUSE 1 : 47 2 : 100	ENTIRE PERIOD		LAST 13 DAYS	
	1	2	1	2
MEAN TEMP.	65	65	70	57
ABS. RANGE	52	60	37	34
AV. DAILY RANGE	21	24	21	22
AV. DAILY MIN.	55	53	60	46

FIG. 2. *Temperature fluctuations during outside rearings of emergent pure lines at Fredericton, N.B., and in central Gaspé, 1935 and 1936. Summary of temperature conditions and percentage of diapause in insets of the figure.*

of the graph. There is no significant correlation between the mean temperatures during the entire period of first and second generation development at Fredericton, and the percentage of insects in diapause. The mean temperatures during the final 13 days of each period, which reflect the late seasonal drop in temperature in the Fredericton district, are more consistently related to the degree of diapause. For a more critical analysis, however, it is instructive to compare temperature conditions during the rearing of the second generation populations at Fredericton with the temperature conditions for the single generation populations in Gaspé. For convenience, the pertinent statistics are shown in the following synopsis.

Year	Statistic	Insects reared in Gaspé	Second generation reared at Fredericton
1935	Basis: the entire period		
	Insects in diapause	44%	100%
	Mean temp.	59	65
	Av. min. temp.	46	53
1936	Insects in diapause	89%	100%
	Mean temp.	56	60
	Av. min. temp.	44	50
1935	Basis: the last 13 days		
	Insects in diapause	44%	100%
	Mean temp.	60	57
	Av. min. temp.	46	46
1936	Insects in diapause	89%	100%
	Mean temp.	57	57
	Av. min. temp.	44	47

Whether comparison be made on the basis of the entire developmental period, or on the basis of the last 13 days, it is evident that a considerable degree of development took place in the Gaspé rearings (July–August) at temperature conditions that were either approximately equal to or less favourable than those at which all the progeny of the Fredericton rearings (August–September) went into diapause. This feature is discussed further in the following section.

INCEPTION OF DIAPAUSE IN FIELD POPULATIONS

Fifth and sixth instar larvae were collected periodically in woodlands in south central New Brunswick and placed in outside cages supplied with foliage and moss. After a short interval the cocoons were removed to wire containers in the soil and subsequent emergence recorded daily. These studies were carried out in three localities for several years, with consistent results. The most complete series were obtained at Young's Brook and Kingsley Road, York County, and the data are summarized in Tables VI and VII. The series for each locality and year showed a progressive decrease in emergence, due to increased incidence of diapause, according to the lateness of larval maturity.

The results of the 1938 Kingsley Road series, with temperature and precipitation records, are shown graphically in Fig. 3. The completeness of the data warrants an analysis of the relationship between weather and the incep-

TABLE VI

SUMMARY OF SUMMER EMERGENCE FROM COCOONS SPUN AT DIFFERENT PERIODS, YOUNG'S BROOK, YORK CO., N.B. (W. A. REEKS)

Lot No.	Time of spinning	No. of cocoons	Emergence, %	Mean date of emergence
1937				
1	June 28 – July 7	19	52.6	July 23
2	July 7 – 23	416	6.0	Aug. 9
3	July 14 – 27	509	6.5	Aug. 17
4	July 20 – Aug. 3	724	3.3	Aug. 16
5	July 23 – Aug. 5	836	3.3	Aug. 18
6	July 28 – Aug. 11	858	1.1	Aug. 21
7	Aug. 4 – 14	624	1.3	Aug. 25
8	Aug. 10 – 23	847	0.9	Sept. 10
9	Aug. 17 – 27	763	0.5	Sept. 15
10	Aug. 25 – Sept. 5	846	0.1	Sept. 28
11	Sept. 2 – 12	645	0.1	?
12	Sept. 8 – Oct. 1	621	0.0	—
13	Sept. 16 – Oct. 7	802	0.0	—
1938				
1	July 14 – 21	198	47.0	Aug. 5
2	July 20 – 28	376	38.6	Aug. 11
3	July 27 – Aug. 4	755	8.9	Aug. 17
4	Aug. 3 – 11	1041	2.6	Aug. 28
5	Aug. 10 – 18	918	0.5	Sept. 10
6	Aug. 17 – 26	757	0.5	?
7	Aug. 27 – Sept. 1	121	0.0	—
8	Sept. 2 – 9	34	0.0	—
9	Sept. 10 – 14	171	0.0	—
10	Sept. 15 – 26	614	0.2	?
11	Sept. 27 – Oct. 11	138	0.0	—
1939				
1	July 11 – 25	129	65.0	Aug. 12
2	July 25 – 31	1128	49.5	Aug. 16
3	Aug. 1 – 8	285	15.4	Aug. 24
4	Aug. 9 – 14	65	18.5	Sept. 1
5	Aug. 15 – 21	40	10.0	Sept. 13

tion of diapause. The analysis may relate to the total period of larval development for each lot, or only to the period of fifth instar development, on the assumption that the latter may be the critical stage in which subsequent developmental behaviour is determined. In either case the appropriate calendar period must be delimited. Since most of the cocoons were spun within a few days of the larval collection date, and since in extensive rearings in south central New Brunswick the total developmental period averaged 30 to 31 days, and that of the fifth instar 13 days, the corresponding periods for each field sample have been approximated as the month and the 13-day period ending with the larval collection date. The analysis has been carried out for alternate sample lots and the statistics appear in Tables VIII and IX.

TABLE VII

Summary of summer emergence from cocoons spun at different periods, Kingsley Road, York Co., N.B. (C. C. Smith)

Lot No.	Time of spinning	No. of cocoons	Emergence, %	Mean date of emergence
1938				
1	June 30 – July 14	4	100	July 22
2	July 6 – 21	396	93.7	July 31
3	July 13 – 28	939	63.3	Aug. 4
4	July 20 – Aug. 4	1647	38.6	Aug. 13
5	July 27 – Aug. 11	1747	16.4	Aug. 20
6	Aug. 3 – 18	2935	5.5	Aug. 26
7	Aug. 10 – 25	1093	1.7	Sept. 25
8	Aug. 17 – Sept. 1	290	2.8	Oct. 16
9	Aug. 24 – Sept. 8	340	1.8	Oct. 18
10	Aug. 31 – Sept. 15	360	0.6	Oct. 16
11	Sept. 7 – 22	640	0.0	—
12	Sept. 14 – 29	416	0.0	—
13	Sept. 21 – Oct. 6	281	0.0	—
14	Sept. 28 – Oct. 13	296	0.3	?
15	Oct. 5 – 20	84	0.0	—
16	Oct. 13 – 27	183	0.0	—
1939				
1	July 4 – 20	90	97.8	July 28
2	July 13 – 27	58	55.1	Aug. 11
3	July 19 – Aug. 3	182	41.2	Aug. 11
4	July 27 – Aug. 9	31	32.3	Aug. 14

TABLE VIII

Rainfall and temperature conditions during the approximate total developmental period of sample lots in the 1938 Kingsley Road series, with percentage of insects in diapause

Lot No.	Approximate total developmental period	Rainfall, in.	Temperature conditions during period*				No. in diapause, %
			Average daily mean temp.	Days with mean temp. 60° or lower	Days with mean temp. 50° or lower	Days with minimum temp. 40° or lower	
1	June 1–30	3.83	64	6	10	0	0
3	June 13–July 13	6.09	64	8	10	0	37
5	June 27–July 27	4.47	66	7	6	0	84
7	July 10–Aug. 10	4.16	70	1	0	0	98
9	July 24–Aug. 24	4.29	70	1	0	0	98
11	Aug. 7–Sept. 7	3.52	63	10	10	3	100
13	Aug. 21–Sept. 21	5.06	59	20	19	7	100

* *Temperature readings (degrees F.) of maximum and minimum thermometers in standard penthouse, twice daily.*

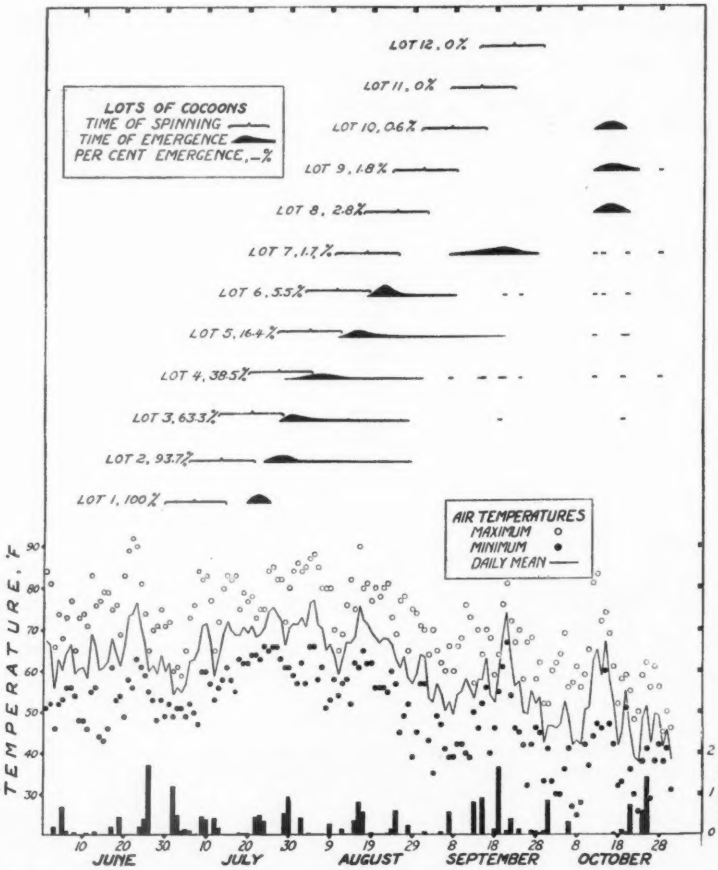


FIG. 3. *Percentage and time of emergence from first generation cocoons spun at successive intervals, Kingsley Road, York County, N.B., 1938, correlated with temperature and precipitation.*

Consideration of these statistics leads to the following conclusions: (1) Least diapause occurred in lots which developed at distinctly less favourable temperature conditions in June and early July, and the rapid increase in diapause was concomitant with a marked improvement in temperature during late July and early August. The slight increase in diapause (2%) in the latest samples coincided with late seasonal decline in temperature, but this has no practical significance in view of the trend established earlier. (2) There was no correlation between mean or minimal temperature within the range experienced, nor between precipitation in the various periods, and the degree

TABLE IX

Rainfall and temperature conditions during the approximate 5th instar developmental period of sample lots in the 1938 Kingsley Road series, with percentage of insects in diapause

Lot No.	Approximate 5th instar developmental period	Rainfall, in.	Temperature conditions during period				Insects in diapause, %
			Average daily mean temp.	Days with mean temp. 60° or lower	Days with mean temp. 50° or lower	Days with minimum temp. 40° or lower	
1	June 18–30	2.64	66	3	3	0	0
3	July 1–13	3.22	62	5	4	0	37
5	July 15–27	1.25	71	0	0	0	84
7	July 29–Aug. 10	1.72	70	0	0	0	98
9	Aug. 12–24	1.89	69	0	0	0	98
11	Aug. 26–Sept. 7	0.82	58	9	10	3	100
13	Sept. 9–21	3.44	59	10	8	3	100

of diapause. (3) Conditions after spinning of the cocoons were not obviously related to diapause, since the greatest increase in the latter occurred during late July and early August, although temperature conditions were equally favourable for about another month and precipitation rate was not significantly different at this period.

One further characteristic, viz., the variability of temperature conditions during larval development, remains to be explored. At this point it is pertinent to refer to Dawson's (33) experiments with Minnesota polyphemus larvae at controlled constant, and varying, temperatures; there was apparently no influence of low constant temperature upon the pupal diapause, but a gradually declining temperature during late larval development seemed to bring on diapause, even though the minimum temperature in the graded series was higher than the lowest temperature in the constant temperature series. Statistics on temperature variability during the fifth instar development of the spruce sawfly in the 1938 Kingsley Road series, are tabulated below.

Lot No.	Av. daily mean temp.	Absolute			Av. daily range	Insects in diapause, %
		Max.	Min.	Range		
1	66	92	48	44	22	0
3	62	83	47	36	18	37
5	71	85	55	30	16	84
7	70	88	51	37	23	98
9	69	90	52	38	20	98
11	58	78	35	43	23	100
13	59	81	39	42	20	100

The degree of variability in temperature was very great, both in the absolute scale and relative to the mean temperature, for Lots 1 to 3 in which diapause was least, and considerably less in the relative scale, though scarcely different in the absolute, for Lots 5 to 7 in which diapause was very high. It appears therefore that variability, per se, in temperature, can have little to do with the inception of diapause in the field populations.

Thus, although it is possible by laboratory experiments to show the influence of new foliage and low variable temperature on the inception of diapause, and although there are distinct differences in temperature conditions between early and late summer associated with absence or presence of diapause in emergent stock, an analysis of the data fails to provide evidence that the inception of diapause in the field population is intimately related to any one of the factors, food, temperature, or precipitation. The success of incubator populations throughout the year might also be taken to indicate the unimportance of sunlight and cyclical changes in the daylight period. It is, however, certain that diapause in emergent stock is determined environmentally, since the phenomenon is eliminated or relatively infrequent in the absence of environmental fluctuations. No one factor is likely to be the sole cause of diapause in *Gilpinia polytoma* and the only conclusion that can be reached is that the insect is evidently highly sensitive to a changing environment, with the consequence that diapause in emergent stock increases progressively with advance of the season.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

II. FACTORS INFLUENCING THE BREAKING OF DIAPAUSE[1]

By M. L. Prebble[2]

Abstract

Field and laboratory experiments have shown the importance of a period of "cold-rest" at a temperature below the threshold of development as a requirement for overcoming diapause in the spruce sawfly, especially in stock from a one-generation area. After cold-rest, maximal development results at a temperature of 74° to 75° F. or higher, and after contact with water. Temperatures in the field are lower and fail to promote so high development as may be obtained in the laboratory; however, temperature variations between 65° and 45° F. evidently have little influence on the degree of emergence from the diapause condition, though speed of development is directly affected. The benefit of contact with water is reduced or lost if contact occurs only while soil temperature remains below the threshold of development, and if the moisture taken up in the cocoon wall is lost by evaporation before it can be absorbed by the larva. The role of the cocoon in water exchanges, and differential effects of abnormal weather conditions upon intracocoon development in stocks in one-generation and two-generation areas, are described.

Approximately 200,000 cocoons have been used in laboratory and field studies of the factors influencing the breaking of diapause. The principal object of the experiments was to provide an understanding of the influence of factors operative in the natural habitat; the effect of artificial stimuli, such as chemicals, etc., has not been investigated. Space limitations require that the discussion be limited to the more important conclusions only, and that only representative tabulations of data be included.

Laboratory Studies

Overwintering cocoons collected in the autumn were divided into sample lots, some of which were opened for immediate analysis while the others were placed in various types of cold storage and periodically incubated under different temperature and moisture conditions. At 70° to 75° F., adults typically began to appear within two or three weeks and continued until the 8th or 10th week. At the conclusion of emergence, the sound cocoons were opened and the number of individuals that died when partially developed (i.e., beyond the eonymph) were added to the number of emerged adults in order to provide a measure of development.

Rest at Low Temperature ("Cold-rest")

In Gaspé cocoons incubated under favourable conditions of temperature and moisture in the early autumn there was only a low degree of development.

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There was no general resumption of development over an indefinite period though many of the eonymphs were apparently healthy after six months or more in the incubator. Development gradually increased in samples left in cold storage for progressively longer periods, reaching a maximum for samples incubated in January or February. Typical results are shown in Table I.

TABLE I

DEVELOPMENT OF GASPÉ COCOONS IN RELATION TO THE DURATION OF COLD-REST

Cocoons were kept in storage in the ground until the dates indicated, then incubated under suitable conditions of temperature and moisture

1933–1934		1934–1935		1935–1936	
Date	Development, %	Date	Development, %	Date	Development, %
Nov. 8	16	Nov. 2	10.0	Nov. 1	2.7
Nov. 18	14	Nov. 16	11.8	Nov. 15	4.2
Dec. 2	21	Nov. 30	21.1	Nov. 29	29.0
Dec. 16	19	Dec. 14	28.8	Dec. 13	40.0
Jan. 2	50	Dec. 28	39.0	Jan. 3	70.8
Jan. 15	51	Jan. 11	30.7	Jan. 20	61.1
Jan. 29	61	Jan. 25	44.5	Jan. 30	65.5
Feb. 13	60	Feb. 8	40.8	Feb. 14	64.8
Feb. 27	71	Feb. 15	46.4	Feb. 22	72.9
Mar. 10	62	Feb. 22	56.0	Mar. 2	64.2
Mar. 24	65	Mar. 1	58.5	Mar. 10	68.5
April 4	64	Mar. 8	49.3	Mar. 18	61.3
April 19	71				

On the other hand, very high development resulted in all series of New Brunswick cocoons incubated at 74° F. and 100% relative humidity from early December onwards. Representative series are summarized in Table II.

TABLE II

DEVELOPMENT IN NEW BRUNSWICK COCOONS SHOWING LACK OF CORRELATION WITH EXTENDED COLD-REST

Cocoons were kept in storage in the ground and incubated on the dates indicated

1938–1939		1939–1940	
English Settlement, York Co.		Kingston, Kings Co.	
Date	Development, %	Date	Development, %
Dec. 13	99.4	Dec. 1	93.6
Dec. 27	99.2	Dec. 20	93.5
Jan. 10	96.5	Jan. 11	92.9
Jan. 24	96.8	Feb. 1	95.2
Feb. 7	99.6		
Feb. 21	99.3		
Mar. 7	98.2		
Mar. 21	98.7		
May 5	97.9		

Temperature during Cold-rest

The results of experiments in which Gaspé cocoons were incubated under favourable conditions of temperature and moisture, after extended cold-rest in various types of storage, are summarized in the following synopsis.

Year	Storage conditions	Development, %
1934–1935 (August collection)	*a.* In ground *b.* 30° to 45° F., av. 35° F. *c.* 35° alternating with 14° F. *d.* 14° F.	46.6 45.2 46.0 42.0
1934–1935 (September collection)	*a.* In ground *b.* 30° to 45° F., av. 35° F. *c.* 35° alternating with 14° F. *d.* 14° F.	52.7 45.0 51.1 22.1
1935–1936	*a.* In ground *b.* 33° to 52° F., av. 40° F. (moist sand) *c.* 33° to 52° F., av. 40° F. (79% R.H.*)	66.1 62.8 72.2

**Throughout the paper R.H. refers to the relative humidity.*

Cold-rest at temperatures continuously above the freezing point was as effective as at fluctuating temperatures in the ground, mostly below the freezing point, or at widely variable temperatures (35° to 14° F.). Although development in the September series of 1934–1935 was uniformly low after storage at 14° F., the August series stored in the same location showed no significant reduction. The results failed to indicate any significant relation between the degree of cold, within these limits, and the resumption of development.

Other sample lots of the August and September collections were incubated at 50° F. in mid-October. There was virtually no emergence, and the samples were analysed after six months, giving the following results.

Collection made in:	Pronymphs, %	Pupae, %	Adults, %
August—			
Analysis in October	10.5	0	0
Analysis after six months at 50° F.	13.6	0	0
September—			
Analysis in October	8.6	0	0
Analysis after six months at 50° F.	8.1	0	0.6

The failure of the overwintering pronymphs to develop at 50° F. can be attributed only to the inadequacy of rest at this temperature for overcoming diapause, since the threshold of development is known to be below 45° F.

It may be inferred that cold-rest, to be effective, must be near or below the threshold of development, a condition that is always realized in the natural habitat during the winter months.

Moisture during Cold-rest

Results of an experiment to determine the effect of contact moisture during extended cold-rest upon subsequent development in the incubator are shown below.

Conditions of storage	Conditions of incubation	
	73° F., 70% R.H.	73° F., moist sand
	Development, %	
Mean temperature 40° F., 79% R.H.	4.8	72.2
Mean temperature 40° F., moist sand	10.2	62.8

With about 700 cocoons in each of the four experimental series, the difference between 4.8 and 10.2 is statistically significant, that between 62.8 and 72.2, marginal. Moisture during cold-rest therefore had a slight stimulating effect on subsequent development where no moisture was provided during incubation, but was without effect in cocoons that were kept moist during incubation. An adequate explanation of the distinction will be found in a later section.

Temperature during Incubation

The results of experiments involving over 5000 cocoons of Gaspé origin and dealing with the relation between incubation temperature and degree of development are summarized in Table III.

TABLE III

PERCENTAGE OF DEVELOPMENT IN RELATION TO INCUBATION TEMPERATURE

All lots had adequate cold-rest, were incubated at 100% relative humidity, and all factors except those noted were identical for the lots of each series

Treatment	Incubation temperature, °F.			
	45°	55°	65°	74 to 75°
	Development, %			
1938–1939				
1. No contact water provided	—	16.0	19.1	94.5
2. Immersed two days in water at 32° to 74° F.	—	24.1	28.6	96.9
1939–1940				
3. No contact water provided	63.7	55.7	71.0	98.8
4. Immersed two days in water at incubation temperature	74.3	58.7	73.0	99.1

The statistical significance of differences between the values may be briefly summed up as follows: Series 1,—16.0 and 19.1, not statistically different one from another, but both different from 94.5; Series 2,—relations as in Series 1; Series 3,—55.7 and 63.7, not different, 55.7 and 71.0, different, 63.7 and 71.0, not different, 98.8, significantly different from all other values; Series 4,—58.7 and 74.3, different, 58.7 and 73.0, different, 74.3 and 73.0, not different, 99.1, significantly different from all other values. Series 3 and 4 therefore provide, unexpectedly, some evidence that an incubation temperature of 55° F. is less efficacious as a stimulus to development than 45° or 65° F., though there is no clear evidence of a difference in the effects of incubation at 45° and 65° F. Series 1 and 2 partly counterbalance the first conclusion, since they fail to indicate significant differences between incubation temperatures of 55° and 65° F. On the whole, the evidence indicates that temperature differences within the limits of 45° and 65° F. have no notable effect upon the degree of development, but a temperature of 75° F. has a markedly more stimulative influence than one of 65° F. or lower.

Development at 45° was very slow, and the first pupae appeared after about seven weeks and the first adults after about 12 weeks, despite the fact that advanced pronymphs were present in the overwintering material at the time of incubation. The threshold of development is obviously only 2° to 3° below 45° F.

Moisture Conditions during Incubation

The importance of suitable moisture conditions as a factor in the resumption of development has been demonstrated for many insects, including the codling moth (5), ragweed borer (2) and the pink bollworm (3, 4), to mention but a few examples. Before describing the effect of moisture upon the resumption of development in the spruce sawfly, it is necessary to remark that divergent results were occasionally obtained when using experimental material of different origin, indicating that uncontrolled factors (physiological state of the overwintering populations) were quite variable. Typical results will be shown and attention drawn to others that were at variance.

Contact Moisture

Contact moisture was supplied by placing the cocoons on moist sand in covered dishes, by frequent momentary dipping, or by immersion for one to three days. Experimental results are summarized in Table IV; the incubator temperature was 74° to 75° F., and all cocoons were held at 100% relative humidity between or after treatments except where otherwise stated.

In general for Gaspé material, there was increased development as the duration of contact with water, or the frequency of dipping, was increased. Infrequent dippings (intervals of four to seven days) were of no benefit when the cocoons were in an unsaturated environment between dippings. Immersion for one to three days was equally effective in promoting development as contact of 14 to 21 days on moist sand, or 14 to 21 daily dippings. Mortality

TABLE IV

PERCENTAGE OF DEVELOPMENT AS INFLUENCED BY CONTACT WATER IN THE INCUBATOR

Year	Origin	Treatment	Development, %
1936–1937	Gaspé	0, check	67.2
		1 day on moist sand	72.0
		3 days on moist sand	72.1
		7 days on moist sand	77.2
		14 days on moist sand	82.5
		21 days on moist sand	93.6
1937–1938	Gaspé	0, check	20.3
		1 day on moist sand	60.6
		3 days on moist sand	83.4
		7 days on moist sand	80.4
		14 days on moist sand	92.4
		21 days on moist sand	88.0
1936–1937	Gaspé (cocoons held at 80% R.H. between and after dippings)	0, check	30.4
		3 dippings, 7-day intervals	23.9
		5 dippings, 4-day intervals	26.5
		10 dippings, 2-day intervals	51.5
		21 dippings, 1-day intervals	57.2
1936–1937	Gaspé	0, check	38.4
		1 dipping	60.8
		3 dippings, daily intervals	81.1
		7 dippings, daily intervals	77.7
		14 dippings, daily intervals	86.3
		21 dippings, daily intervals	91.7
1936–1937	Gaspé	0, check	38.4
		1-day immersion	77.1
		2-day immersion	80.4
		3-day immersion	80.9
1937–1938	Gaspé	0, check	20.3
		1-day immersion	89.0
		2-day immersion	94.5
		3-day immersion	95.6
1938–1939	English Settlement, N.B.	0, check	98.8
		2-day immersion	99.0
1938–1939	Douglas Harbour, N.B.	0, check	96.5
		2-day immersion	98.2
1939–1940	Kingston, N.B.	0, check	93.8
		2-day immersion	90.3

in the various developmental stages was frequently less after immersion for short periods than after the more prolonged contact with water.

Exceptional results were obtained in the 1938–1939 and in the 1939–1940 experiments with Gaspé material, equally high development resulting in cocoon samples incubated at 100% relative humidity, 74° to 75° F., whether or not contact water was supplied. It should also be noted that contact water at incubator temperatures of 45° to 65° F. has a reduced and doubtfully significant effect, indicating that the response to water addition is largely conditioned by incubation temperature.

In contrast with typical Gaspé material, all series of New Brunswick samples incubated at 74° F., 100% relative humidity, gave uniformly high development independent of contact water.

Temperature of Water during Immersion

Cocoons were immersed for two days in water at various temperatures from 32° to 86° F., and samples from each immersion temperature were then incubated at 100% relative humidity and at 55°, 65°, and 74° F. Development in the various samples, consisting of 100 to 150 cocoons each, is summarized below.

Immersion temperature, °F.	Incubation temperature, °F.		
	55°	65°	74°
	Development, %		
1. Check (not immersed)	16.0	19.1	94.5
2. 32°	24.7	27.9	96.4
3. 40°	25.7	27.4	96.4
4. 55°	21.8	—	96.5
5. 65°	20.6	25.5	97.2
6. 74°	27.7	33.6	97.8
7. 86°	97.2	98.5	87.1
Average, Series 2 to 6	24.1	28.6	96.9

The effect of the immersion temperature was partly obscured by the high development in all samples incubated at 74°. However, immersion at 86° greatly increased development in the samples incubated at 55° and 65°. In analysing the differences between the checks, and the combined values for all samples immersed at temperatures of 32° to 74°, for each of the incubation temperatures of 55° and 65°, the probability values are 0.07 and 0.05 respectively, indicating but not definitely proving that development was higher as a consequence of immersion, whereas the variations in the degree of development in samples immersed at temperatures between 32° and 74°, were for each incubation series at 55° and 65°, not greater than would occur as chance fluctuations, the probability in each case being approximately 0.75.

One may conclude from this experiment that the temperature of water during contact, within the limits of 32° and 74°, is of no importance, the essential requirement being the addition to the cocoon wall of water that is subsequently utilized by the contained larva if environmental conditions permit. These conditions include a temperature favourable to development and a humid external environment so that the moisture gained in contact is not lost by evaporation.

Atmospheric Humidity

Experiments were conducted to determine the influence of atmospheric humidity on degree of development when the cocoons were or were not given previous contact with water, and also when the contact with water was subsequent to various degrees of drying. In the experiments summarized in the following synopses, all samples had adequate cold-rest to ensure high development at suitable conditions, and all lots were incubated at 73° to 74°; contact with water was also at the same temperature.

a. Cocoons incubated without contact water.

1. Gaspé material, 1935–1936; overwintering pronymphs about 3% of the population.

R.H. in incubator, %	5–10	10	26	55	81	100	Check, moist sand
Development, %	5.3	7.2	4.3	5.5	8.0	38.1	65.0

2. Gaspé material, 1936–1937; overwintering pronymphs about 6% of the population.

R.H. in incubator, %	20	56	81	100	Check, moist sand 21 days, then 100% R.H.
Development, %	17.5	18.4	40.9	67.2	93.6

3. Gaspé material, 1937-1938; overwintering pronymphs about 3% of the population.

R.H. in incubator, %	15	45	81	100	Check, 21 daily dippings, then 100% R.H.
Development, %	2.7	4.7	7.7	20.3	82.7

b. Cocoons placed on moist sand for various periods, then transferred to different conditions of atmospheric humidity.

Gaspé material, 1936-1937; overwintering pronymphs about 6% of the population.

Initial period on moist sand	R.H. in the incubator, %			
	20	56	81	100
0, check	17.5	18.4	40.9	67.2
1 day	22.4	23.5	57.1	72.0
3 days	23.1	26.8	63.0	72.1
7 days	24.0	41.7	68.8	77.2
14 days	45.2	57.6	87.4	82.5
21 days	65.2	74.7	86.5	93.6

c. Cocoons held at 15% R.H., 74° F., for various periods, then transferred to 100% R.H. with or without immersion after the initial drying.

Gaspé material, 1937–1938; overwintering pronymphs about 3% of the population.

Period of initial drying (weeks)	Samples with no contact water at transfer	Samples immersed for two days at transfer
0, check	20.3	94.5
1	10.8	81.9
2	10.8	71.2
4	6.9	31.5
6	5.1	9.0
8	2.7	5.6

Bearing in mind that a small proportion of pronymphs was present in each experimental population, and that this must be considered in judging the influence of the various treatments, the experimental results warrant the following conclusions: (1) Development was inhibited in all unsaturated environments, but was not prevented altogether even in the driest environment; (2) the benefit of an initial contact with water for varying periods was reduced in relation to the degree of unsaturation of the environment to which cocoons were later exposed; (3) drying of the cocoons reduced the capacity for development when drying ceased, and also reduced the capacity to benefit from subsequent contact with water, in relation to the amount of initial drying, since there was practically no response after drying for eight weeks at 15% relative humidity, 74° F.

CRITICAL PERIOD

Rice (2) found that while emergence of *Epiblema strenuana* from its larval diapause was promoted by frequent wettings, the timing of the contact with water was extremely important. If the wettings were discontinued before the normal period of pupation, or were not started until several weeks afterwards, there was a marked reduction in the response to the same schedule of wettings. This indicated a critical period, corresponding to the normal pupation time in nature, at which the insect was most sensitive to moisture addition.

It has already been shown that the response of spruce sawfly material from Gaspé, to identical temperature and moisture conditions, becomes increasingly greater with cold-rest extending to January or February. The response at an incubator temperature of 74° is much greater than that which occurs in nature in the following summer. Repeated experiments, in which successive samples of cocoons were incubated under the same conditions at various periods during the late winter and the spring, have failed to provide any convincing evidence that the sensitivity of the insect to moisture addition varies in relation to the natural period of development. The difference in the degree of development realized in the incubator and in the field can therefore be attributed only to the differences in temperature and moisture conditions between the two environments.

INFLUENCE OF THE COCOON ON WATER EXCHANGES

The function of the cocoon in maintaining the micro-climate has been investigated by Ullyett (6) in studies of the physical ecology of *Microplectron fuscipennis* as a parasite of *Diprion sertifer.* From determinations of parasite development and survival at various saturation deficiencies of the external environment, and comparison of weight changes of naked and cocooned host larvae, Ullyett concluded that the micro-climate was kept near the saturation point by moisture from the host, and hence could not absorb moisture contained in the cocoon wall. The protective influence of the cocoon broke down only in very dry air, much more extreme than would normally be encountered in the natural habitat.

The problem of determining the role of the cocoon in regulating water exchanges in *Gilpinia polytoma* was of particular importance because of the relationship between moisture conditions of the external environment and the resumption of development.

Loss of Water

Studies of water loss in Gaspé cocoons containing a minimum (2 to 3%) of overwintering pronymphs, were carried out sufficiently early in the cold-rest period to avoid development within the cocoons during the progress of the experiments. The gross weight of 12 samples, each of 50 cleaned cocoons, was determined, and six samples were placed in desiccating jars at 75° F. The other six samples were dissected, the weight of the larvae and of the cocoon shells determined, then they too were placed in the desiccating jars. Weight determinations of the sound cocoons, naked larvae, and empty shells were made periodically to the 40th day, and then all larvae were brought to constant weight in the oven.

Data necessary for the interpretation of the experimental results are included in the synopses.

a. Relation between gross weight and net larval weight at the start of the experiment (All weights in centigrams)

Sample	Gross weight	Larval weight	Larval weight as percentage of gross weight
1	360.2	311.1	86.37
2	358.3	308.9	86.21
3	366.6	315.8	86.14
4	370.5	318.9	86.07
5	375.3	322.9	86.04
6	364.1	314.5	86.38
Totals	2195.0	1892.1	86.20

b. Weight changes in naked larvae in 40 days

Average initial live weight	Saturation deficiency	Average weight at at end	Average oven-dry weight	Water content, %
6.22	22.0	1.98	1.97	68.3
6.18	16.5	1.98	1.95	68.4
6.32	12.8	2.04	1.97	68.8
6.38	8.4	2.12	1.98	69.0
6.46	4.2	2.37	2.03	68.6
6.29	0	—	—	—
Average of all lots: 6.31			1.98	68.6

c. Final weight of larvae left in the cocoons until the end of the experiment

Saturation deficiency	Average live weight at end*	Average oven-dry weight
22.0	4.55	1.93
16.5	4.92	1.98
12.8	4.98	1.92
8.4	5.41	1.98
4.2	5.69	1.96
0	6.19	1.99
		Average of all lots: 1.96

* *There was no mortality of larvae during the experiment.*

The larvae constituted 86.2% of the initial gross weight, had an average live weight of 6.31 cg., and a water content of 68.6% of the live weight—assuming that weight loss during drying was entirely due to water loss. That this assumption is essentially correct is indicated by the fact that the average oven-dry weight (1.97 cg.) of the naked larvae at a saturation deficiency of 22.0 mm., where death supervened within a few days, was not significantly different from the oven-dry weight of naked larvae (1.95 to 2.03 cg.) in the more humid environments where death came gradually, or from the oven-dry weight of the cocooned larvae (1.96 cg.) which were all alive at the end of the experiment. It may be concluded that weight loss due to oxidation of reserve substances was negligible, and hence that the weight loss was virtually all due to loss of water.

The water loss from sound cocoons (Fig. 1, top panel) at each saturation deficiency was greatest during the first two days, and gradually approached direct proportionality to time. In the strict sense, however, the regression of water loss on time was slightly curvilinear over the entire 40-day period. The slight loss of water in the so-called saturated environment was apparently due to the periodic disturbance at the successive weighings. The ultimate oven-dry weight of the contained larvae (1.99 cg.) was exceeded by only one other sample, so the loss from the sound cocoons in so-called saturated environment cannot logically be attributed to loss of reserve substances through metabolism.

Naked larvae lost water rapidly, almost independently of saturation deficiency within the limits of 4.2 to 22.0 mm. (Fig. 1, middle panel), and the loss in 40 days in the driest environments was essentially the entire water content. In the dry environments, the first deaths occurred when water loss was about 40%, and all larvae were dead when the loss reached 60% of the initial live weight. There was also a considerable loss of water in the humid environment; the first mortality occurred when water loss was 10%, and all larvae were dead in 12 days with an average loss of 23.12%, and were discarded because of mold.

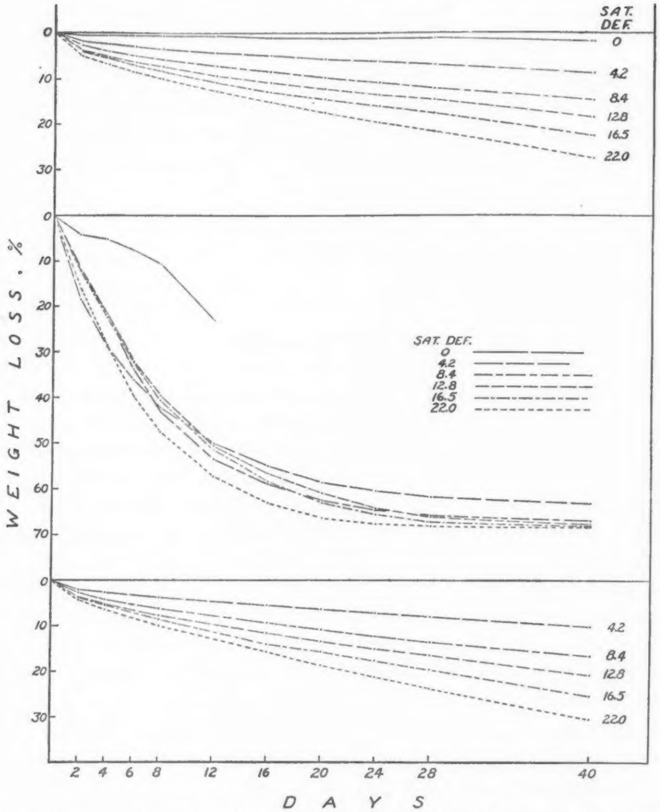

FIG. 1. *Percentage loss of weight (water) of cocoons and naked eonymphs in relation to time and saturation deficiency at 75° F. Top panel, sound cocoons with contained eonymphs; middle panel, naked eonymphs; bottom panel, loss from eonymphs within sound cocoons (see text).*

Part of the water loss from sound cocoons relates to moisture in the cocoon wall, and the balance to moisture from the contained larva. It is possible to deduce the amount attributable to the larva alone, using the method of Ullyett. For each determination of gross weight loss, there was a corresponding determination of loss from the empty shells, and since the latter constituted 13.8% of the initial gross weight, the proportion of gross weight loss attributable to shells, and hence also that attributable to larvae, can readily be calculated. The following example, for 12.8 mm. saturation deficiency and a period of 40 days, will make the method clear.

Loss of initial gross weight = 18.64%

Loss of initial cocoon shell weight = 4.92%

Loss from shells $= 4.92 \times 0.138$
$= 0.68\%$ of initial gross weight

Balance of gross weight loss attributable to contained larvae
$= 18.64 - 0.68$
$= 17.96\%$ of initial gross weight

This loss, in terms of *initial larval weight* $= 17.96 \div 0.862$
$= 20.83\%$

The estimated water loss from the protected larvae in cocoon samples at saturation deficiencies of 4.2 to 22.0 mm. and for periods of 2 to 40 days, is shown graphically in the bottom panel of Fig. 1. The percentage loss from protected larvae was at first less than the percentage loss of gross weight, but after four to eight days the relation was reversed. This was of course due to the early withdrawal of all moisture from the cocoon wall, all subsequent loss arising solely from the larvae and being naturally a larger proportion of larval weight than of gross weight. The regression of water loss on time for the protected larvae was also slightly curvilinear.

It is also of interest to enquire into the relation between water loss and saturation deficiency for a given time interval. The determined water loss from sound cocoons is plotted against saturation deficiency for four time intervals in the top panel of Fig. 2; the bottom panel contains similar data for the estimated water loss from the contained larvae. Although the plotted linear regression lines (from the formula, $Y = a + bX$) give a fairly good fit for saturation deficiencies between 4.2 and 22.0 mm., the true regression is slightly curvilinear, passing through the origin.

The curvilinearity of the regression, first, of water loss on time at a given saturation deficiency, and second, of water loss on saturation deficiency for a given time, indicating a gradually declining water loss in relation to period and degree of drying, may be due to a decrease in permeability of the cocoon wall consequent upon drying. It can hardly be due to any intrinsic property of the larvae, since when exposed they lose water rapidly in only moderately dry air.

Although the experimental results confirm the opinion reached by Ullyett that the cocoon is very important in the conservation of moisture (being, in *Gilpinia polytoma*, apparently the only mechanism possessed by the larva for this purpose), they show that the cocooned larva is far from being independent of the external environment. The micro-climate appears to be kept near the saturation point by moisture from the larva, as only this condition can account for the water loss at the most humid environments used in the experiments; the maintenance of the humid micro-climate, however, is accomplished at the expense of the insect, since in dry air the cocoon only delays but does not prevent a continuous loss of water from the tissues.

Gain of Water

Experimental results already described have shown that larvae within the cocoons benefit from water gained at contact only gradually, and to a reduced

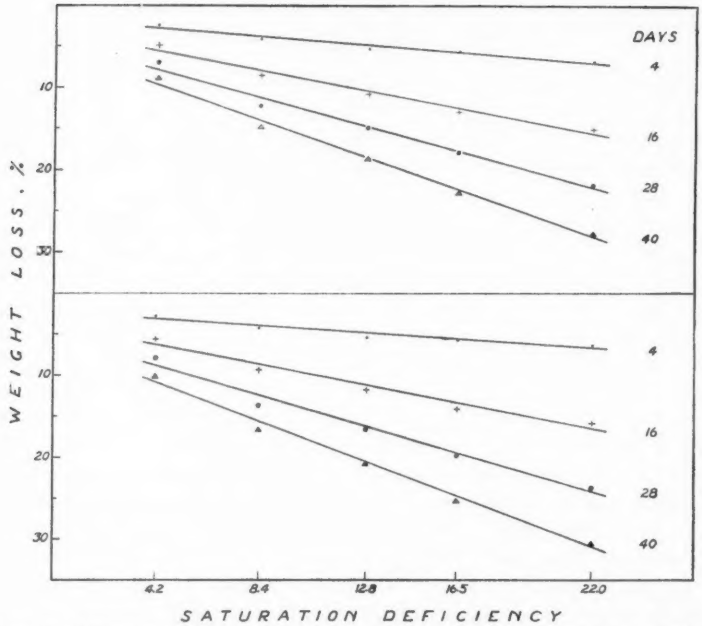

FIG. 2. *Regression, weight (water) loss on saturation deficiency, for four time intervals at 75° F. Top panel, sound cocoons with contained eonymphs; bottom panel, the contained eonymphs alone.*

extent or not at all if the water is rapidly lost by evaporation, and that drying reduces either the permeability of the cocoons to water, or the ability of the larvae to benefit from water gained subsequent to drying.

The outer wall of cocoons in contact with water becomes saturated within a short time. In this condition a structure not readily observed in the dry state may be detected, namely, an inner transparent membranous lining (possibly originating as a liquid secretion from the spinning larva, hardening on exposure). The lining is less pervious than the outer wall, and only minute droplets of water penetrate to its inner surface after contact for a day or longer. If wetted cocoons are placed in a saturated environment the minute droplets gradually disappear, and since here the external environment has no evaporative power, one must presume that the droplets are absorbed by the larvae. Two experiments described in the following paragraphs were designed to test this conclusion by means of weight and water content determinations of larvae after immersion of cocoons.

It was impossible of course to obtain consecutive measurements for the same larvae before and after immersion, so that comparisons had to be made between sample lots from a population. The pre-immersion weight of larvae extracted from the cocoons after immersion was deduced by means of determined relations between gross weight and larval weight. Precautions to reduce errors arising through evaporation from the cocoons and larvae during dissection and weighing, included the use of covered weighing dishes with a very small aperture, and, where only live weights were required, the addition of a non-volatile oil to the weighing dishes, which prevented all moisture loss from the submerged larvae.

The first experiment involved live-weight determinations of larvae extracted immediately after a one- or two-day immersion of the cocoons at 75° F. Some of the cocoons were immersed without previous drying, whereas others were dried (75° F., 22.0 mm. saturation deficiency) for periods of 3 to 56 days before immersion. In all, 28 samples of 100 cocoons each were used; eight samples were opened at the start to determine the initial relationship between gross weight and larval weight, and one sample was opened after each period of drying to determine the then existing relation between gross and larval weight. A summary of the experimental results appears in Table V.

TABLE V

RATIO OF LARVAL WEIGHT TO GROSS WEIGHT, AND LOSS OF WEIGHT (WATER) FROM CONTAINED LARVAE IN RELATION TO PERIOD OF DRYING; COMPARISONS OF THE DETERMINED LARVAL WEIGHTS OF DIFFERENT SAMPLES; AND COMPARISONS OF THE DETERMINED LARVAL WEIGHT AFTER IMMERSION WITH THE DEDUCED LARVAL WEIGHT BEFORE IMMERSION, FOR INDIVIDUAL SAMPLE LOTS

Period of drying in days	0	3	7	14	28	42	56
1. Larval weight as percentage of gross weight	84.92	86.51	86.12	85.21	84.23	83.40	82.19
2. Estimated weight loss from larvae, %	0	4.24	7.67	11.84	20.72	25.64	32.51
3. Determined average larval weight:							
a. Samples not immersed	6.28	5.96	5.70	5.48	4.90	4.70	4.31
b. Samples with 1-day immersion	6.19	5.98	5.91	5.58	5.06	4.64	4.31
c. Samples with 2-day immersion	6.32	6.03	5.92	5.44	5.09	4.67	4.21
4*a.* Samples with 1-day immersion:							
Estimated weight before immersion	6.19	5.93	5.79	5.52	4.94	4.64	4.30
Determined weight after immersion	6.19	5.98	5.91	5.58	5.06	4.64	4.31
4*b.* Samples with 2-day immersion:							
Estimated weight before immersion	6.28	5.98	5.81	5.41	4.93	4.67	4.24
Determined weight after immersion	6.32	6.03	5.92	5.44	5.09	4.67	4.21

NOTE: *Samples whose average weight is underlined show no evidence of an increase as a consequence of immersion.*

A simple method of estimating the effect of immersion is to compare the weights of various sample lots, as in Panel 3 of Table V. There is evidence of gain of water consequent upon immersion in eight of the 10 samples in which weight loss (water) during the earlier drying had not exceeded 21%.

This method is obviously crude since it ignores known differences in original gross weight of the various samples.

A more accurate estimate is obtained by deducing larval weight in a particular sample immediately before immersion. This may be done in two ways, as illustrated for the sample immersed for one day after a 3-day period of drying.

(*a*) Average initial gross weight $\doteq$ 7.275 cg.
Average initial larval weight = 7.275 × 0.8492
= 6.18 cg.
Loss of weight during 3-day drying = 4.24%
Therefore, larval weight prior to immersion = 5.92 cg.

(*b*) Average gross weight after drying = 6.87 cg.
Ratio, larval weight to gross weight after 3-day period of drying
= 86.51%.
Therefore, larval weight prior to immersion = 5.94 cg.

The average of the two estimates, 5.93 cg., is the best approximation of the true larval weight of this sample prior to immersion. All estimated larval weights in Panels 4*a* and 4*b* of Table V have been calculated in this manner. Nine of the 10 samples in which weight loss during earlier drying had not exceeded 21%, showed evidence of a gain during immersion.

It is of interest that immersion for two days had no appreciably greater effect, as measured by these methods, than immersion for a single day. Both failed to compensate at once for a previous loss as low as 4.2% of the initial weight.

The second experiment involved water content determinations of eonymphs in samples analysed immediately before and after, and periodically after immersion for two days at 74° F. Samples analysed at successive intervals after immersion were held meanwhile at 100% relative humidity. Four replications each with over 500 eonymphs, were carried out to determine whether the capacity of the larvae to absorb water was influenced, first, by preliminary drying, and second, by the duration of cold-rest. The data (Table VI) lead to the following conclusions: (1) The water content of eonymphs extracted from cocoons immediately after immersion was not consistently different from that prior to immersion; (2) the water content in samples analysed some days after immersion was in all replications greater than that prior to immersion, and in three of the four replications was greatest in the latest sample after immersion, indicating that absorption of water was gradual; (3) there was no evidence of a relation between duration of cold-rest and ability to absorb water; and (4) eonymphs that had lost about 8 to 9% of their contained water during earlier drying failed to compensate for this loss during two days' immersion and 16 days' storage at 100% relative humidity.

TABLE VI

RATIOS OF WEIGHT OF WATER TO DRY WEIGHT IN SAMPLES OF EONYMPHS EXTRACTED FROM COCOONS BEFORE AND AFTER IMMERSION FOR TWO DAYS AT 74° F.

Replication	1*	2**	3**	4**
Date started	Nov. 21	Dec. 6	Feb. 6	April 7
Initial determination	1.854	1.844	1.868	1.860
Determination after drying	1.703	–	–	–
Determination directly after immersion	1.701	1.863	1.857	1.878
Determination after:				
a. 2 days at 100% R.H.	1.744	1.884	1.939	1.918
b. 4 days at 100% R.H.	1.754	1.862	1.908	1.901
c. 8 days at 100% R.H.	1.751	1.898	1.902	1.898
d. 12 days at 100% R.H.	1.737	1.902	1.900	1.890
e. 16 days at 100% R.H.	1.766	1.948	1.930	1.937

* *All samples in the first replication except those analysed in the initial determination, were partially dried for one week at 18% relative humidity, 74° F., before being immersed.*

** *The other three replications differed from the first only in the absence of a period of drying.*

Ecological Significance.

The cocoon, though indispensable for the conservation of the water supply of the contained eonymph, merely delays the outward diffusion of moisture that occurs in unsaturated environments. The final result for the larva is essentially the same as for many entirely unprotected species in which water loss is proportional to saturation deficiency (1). The inner membranous lining of the cocoon is instrumental in preventing flooding of the microclimate. The space within the cocoon does not become flooded even after several weeks' immersion, and though death from suffocation may result in a week or so at 75° F., eonymphs can survive immersion for several weeks in cold water. Moisture penetrating through the cocoon wall is gradually absorbed by the eonymphs, but may also be very quickly lost by evaporation into the surrounding atmosphere. Preliminary drying of the cocoons may cause a greater water loss than can be compensated for by immersion and a subsequent period of two weeks in saturated environment.

The ecological significance of these facts relates to the need for contact water and a fairly extended period during which the natural habitat is at or near the saturation point, following the period of cold-rest, in order that the maximal development in the overwintered cocoons may be realized. The absence of soil moisture, especially if accentuated by unusually warm weather in the spring or early summer, may have consequences on seasonal development extending well beyond the first rainfall.

Field Studies

Temperature

Sample lots of cocoons collected in central Gaspé in the spring, soon after the disappearance of the snow, were placed for the seasonal development in various sites representing the full range of variability in temperature conditions available in the Gaspé forest. Experimental results for three series are shown in Table VII. Although speed of development was clearly influenced by temperature in each location, as evidenced by the mean dates of adult emergence, in no case was there a significant difference in the percentage of development within the samples from a common population.

TABLE VII

PERCENTAGE OF DEVELOPMENT AND MEAN DATE OF EMERGENCE IN SAMPLES OF THREE OVERWINTERED GASPÉ COCOON POPULATIONS PLACED UNDER DIFFERENT CONDITIONS IN THE FOREST FOR SEASONAL DEVELOPMENT

Year	Forest conditions	Mean Temperature*	Number of cocoons	Development, %	Mean date of emergence
1933	Forest floor, valley bottom	?	288	32.6	Aug. 3
	Forest floor, western slope	48°	289	33.2	July 28
	Seven feet from ground, in shade	55°	296	31.1	July 7
1934	Basement, log building	49°	596	10.2	July 25
	Forest floor, western slope	49 to 50°	580	11.2	July 22
	Seven feet from ground, in shade	53°	583	10.1	July 6
1935	Basement, log building				
	a. Moist sand	52°	402	14.2	July 16
	b. Atmospheric humidity	52°	248	16.5	
	Forest floor, western slope				
	a. Moist sand	53°	387	15.0	July 12
	b. Atmospheric humidity	53°	412	15.5	
	Four feet from ground, in shade				
	a. Moist sand	59°	408	15.7	July 3
	b. Atmospheric humidity	59°	412	16.5	

* *Mean temperature in actual location of cocoons during June and July.*

An experiment involving a much wider difference in temperature was conducted in 1938. Cocoons collected in two localities in central Gaspé were divided into samples for seasonal development, first, in the natural habitat where mean temperature during June–July was 52° F., and second, in the incubator at 74° F., 100% relative humidity. The results follow:

Locality	Collection date	Location of seasonal development	Number of cocoons	Development, %
Berry Mt. Brook	June 2	Forest floor	770	7.1
	June 13	Forest floor	625	5.3
	June 13	Incubator	434	14.1
Brandy Brook	June 2	Forest floor	917	8.9
	June 13	Forest floor	752	6.5
	June 13	Incubator	380	15.5

Development in the incubator samples was significantly higher than in the field samples, by about 8% on the average, a rather small difference in view of the 22° temperature difference between the two environments.

The results of the field experiments, considered in conjunction with those of the laboratory experiments already described, warrant the conclusion that variations in temperature within the limits normally experienced in the natural habitat of the cocoons have no direct influence upon the degree of development in overwintered cocoon populations.

Moisture

Investigations on the influence of moisture upon development under field conditions fall into three groups, first, moisture conditions controlled; second, effects of rainfall; and third, analysis of weather conditions associated with abnormal development.

Moisture Conditions Controlled

Sample lots of cocoons collected in central Gaspé in September, 1935, were overwintered in wire "flats" under the moss, fully exposed to moisture in the natural habitat; other samples were overwintered in inverted vials so located under the moss that the cocoons were protected from moisture. Early in June half of the samples from each group were placed on moist sand in covered dishes for seasonal development, and the other half were placed in dishes without moisture. All samples were subject to the same temperature fluctuations throughout the year. The remaining cocoons were opened for analysis in the fall of 1936, and the results shown in the accompanying synopsis are based on the number of living larvae at the start of the 1936 season.

Overwintering conditions	No moisture in summer		On moist sand in summer	
	Number of cocoons	Development, %	Number of cocoons	Development, %
Protected from moisture	724	7.5 (4.4–10.5)	793	17.2 (14.9–19.3)
Exposed to moisture	541	9.8 (4.9–13.2)	530	18.9 (16.0–21.3)

(Values in parentheses indicate the range in the four samples in each series.)

Moisture during the overwintering period had no influence on seasonal development, while contact moisture supplied from early June increased development significantly, by about 9% on the average.

The results of an experiment on the influence of moisture on seasonal development of Gaspé cocoons in 1935, are summarized in the lower part of Table VII. It has already been noted that temperature differences between the three sites had no effect upon the degree of development, and it is equally clear that the addition of moisture during the summer was without any influence in either of the three sites.

Samples of Gaspé cocoons collected from water saturated moss, June 8, 1936, were placed under various conditions of moisture ranging from moist sand to dry air. With the exception of the third sample (forest floor), all lots were subject to air temperature in the spruce forest. Development in the various samples was as follows:

Lot	Moisture conditions	Number of cocoons	Development, %
1	Moist sand	151	41.1
2	100% R.H.	198	36.9
3	Near 100% R.H. (forest floor)	228	28.1
4	80% R.H.	189	30.7
5	39% R.H.	169	26.6
6	15% R.H.	197	28.9

A Chi-square test of independence of the entire series shows that equal variations might have occurred due to chance alone in about 14% of trials. When the test is between Lot 1, as a treated sample, and the combined results

TABLE VIII

THE INFLUENCE OF RAINFALL ON DEGREE OF DEVELOPMENT

Locality	Year	Protected from rainfall		Exposed to rainfall	
		Number of cocoons	Development, %	Number of cocoons	Development, %
Brandy Brook, central Gaspé	1939	941	17.2 (16.6)	1012	29.2 (21.8)
Berry Mountain Brook, central Gaspé	1939	1102	19.5 (18.2)	1070	28.8 (22.7)
Cascapedia, southern Gaspé	1939	497	46.4	514	61.2
Matapedia, southern Gaspé	1939	288	61.8 (56.6)	314	69.4 (61.8)
St. Leonard, northern New Brunswick	1939	231	37.3 (36.4)	196	50.0 (46.4)
Acadia Expt. Station, central New Brunswick	1938	548	26.8 (26.2)	148	70.9*
Acadia Expt. Station, central New Brunswick	1939	758	53.6 (52.5)	865	77.0 (73.4)
Millville, central New Brunswick	1938	736	41.3 (31.8)	3483	92.4 (90.3)
English Settlement, Central New Brunswick	1939	2598	32.5 (31.0)	4775	88.9 (86.4)

* *Based on emerged adults only, therefore conservative.*

of Lots 2 to 6, as an untreated sample, with development values of 41.1 and 30.3, respectively, the probability of a chance variation of equal magnitude is only about 0.04, giving good evidence that water addition caused increased development. When the test is between Lots 2 to 6, the probability of chance variations of equal magnitude is 0.41, precluding an opinion that the degree of atmospheric humidity was significantly related to the degree of development.

Rainfall

The influence of summer rainfall upon percentage of development was checked by exposing spring collected samples of overwintered cocoons to the rainfall that penetrated through the moss into the wire containers, and by protecting other samples by a waterproof cover. Experiments were carried out in eight localities in Gaspé and New Brunswick; the results are shown in Table VIII.

Rainfall data for the localities during the period of development are included in the synopsis.

	May	June	July	Aug.	Sept.
Central Gaspé, 1939	—	3.04	10.25	10.64	4.19
Northern New Brunswick and southern Gaspé, 1939*	—	3.3	6.8	5.6	5.1
Central New Brunswick (Fredericton), 1938	4.42	3.83	5.89	3.32	4.77
1939	2.42	2.00	2.70	1.04	3.89

* *Average of six weather record localities.*

Two values appear under the percentage development in Table VIII. The higher value, including young pronymphs whose reactivation occurred late in the season, though accurate as a measure of the number of individuals overcoming diapause, is somewhat too large as a measure of effective development during the season. A more accurate expression of the latter is given by the value in parenthesis, from which young pronymphs found in the autumn have been excluded. On the basis of the latter values, effective development in central Gaspé in 1939 was increased significantly, by about 4 to 5%, due to the heavy rainfall during the summer. The results for Cascapedia, Matapedia, and St. Leonard were suggestive of the influence of rainfall in increasing development, but the samples were rather small and the differences without statistical significance except in the case of the Cascapedia samples. The four series in central New Brunswick gave large and significant differences in development due to rainfall during the season, even though the rainfall was less abundant than that experienced in the more northerly localities where the effects on development were of a lower order.

Weather Associated with Abnormal Development

Three instances of abnormal development falling within our experience warrant a brief description.

Phenomenally low emergence, of only 1 to 1.6% as determined from records and analysis of over 6300 field collected cocoons, occurred at Parke Reserve, Kamouraska County, Que., in 1934. The emergence occurred between mid-June and late July, and there was no resumption of development among the eonymphs in diapause later in the season. Partial weather records for Parke Reserve, and complete data for the nearest permanent station of the meteorological service (Ste. Anne de la Pocatière) appear in the synopsis.

	May	June	July	Aug.
Parke Reserve				
Mean temperature	—	56.3	61.2	57.5
Rainfall	—	4.67	3.29	2.92
Ste. Anne de la Pocatière				
Mean temperature	52.2	59.0	65.6	61.6
Departure from normal	+2.7	+1.1	+1.0	+0.7
Rainfall	1.85	4.87	2.94	3.77
Departure from normal	−1.38	+1.86	−0.52	+0.45

There was a marked deficiency of rainfall in May, while later in the season rainfall was close to, or above, normal. The deficiency in May, which was associated with high temperature, was accentuated by the open growth and shallow moss layer in the forest, encouraging the rapid drying out of the debris sheltering the cocoons. The failure of the population to respond to liberal moisture in June and later was possibly due in part to consequences of the earlier drying, though it was typical of the normal behaviour in a one-generation area.

The effect of dry weather in 1938 and 1939 on the overwintered population in the two-generation area in south central New Brunswick was of an entirely different character from that noted above. Normally, emergence from the overwintered cocoons in this region is completed in early July, but in 1938 and 1939 there was a period of heavy emergence in late May through June, and a straggling emergence lasting until October. Many partially developed insects were still inside the cocoons in the autumn. The total seasonal development, however, approximated that which more typically occurs from May to early July.

Climatic data for the two seasons follow (Fredericton records).

	May	June	July	Aug.	Sept.
1938—					
Mean temperature	49.3	64.3	67.1	67.2	55.9
Departure	−1.6	+4.1	+1.1	+3.1	−0.1
Rainfall	4.42	3.83	5.89	3.32	4.77
Departure	+1.29	+0.13	+2.33	−0.66	+1.27
1939—					
Mean temperature	50.0	59.5	67.6	68.6	56.2
Departure	−0.9	−0.7	+1.6	+4.5	+0.2
Rainfall	2.42	2.00	2.70	1.04	3.89
Departure	−0.71	−1.70	−0.86	−2.94	+0.39

The monthly summaries for 1938 show no apparent cause of protracted development, since rainfall was abundant during the early summer months. Actually, over 95% of the May precipitation occurred during the first three weeks when air temperature was low and when soil temperature in the spruce woodlands was almost continuously below the threshold of development. Following this there was a period of unseasonably warm weather with only 1.78 in. of rainfall from May 22 to June 23, the period at which development within the overwintered cocoons generally is most active.

In 1939 there was a scarcity of rainfall in south central New Brunswick from May until September. Moreover, the May rainfall was of little benefit to the sawfly since it occurred when the soil temperature was below the threshold of development practically every night, and above it to the extent of only a few degrees for a limited period during the warmest part of the day. The forest floor was very dry during the gradual rise of soil temperature in June.

Experiments already described leave no doubt that the gradual response of eonymphs during the summer and early fall was due to the effect of delayed rainfall. It is of interest to note that while development in the absence of contact water in the natural habitat remained low, high development resulted in samples incubated without contact water at 74°, 100% relative humidity (e.g., 32.5 and 98.5% development, based on 2598 and 623 cocoons, respectively, in English Settlement samples). The constant high temperature of the incubator therefore provided a stimulus to development in this two-generation material equivalent to that provided in nature by precipitation during the summer.

Reviewing the evidence regarding the influence of moisture, it is fairly clear that moisture conditions during the winter months, or prior to the spring rise in temperature above the threshold, have little effect upon seasonal development in the natural populations. Moisture deficiency at the time of normal spring development is likely to have adverse effects, which, however, are different in one-generation and in two-generation areas. In a two-generation area many of the overwintered eonymphs that fail to develop at the normal time respond to moderate rainfall later in the season, so that the chief result of dry spring weather is a protraction of the emergence period. As for one-generation areas, although continual contact with moisture from early June increased development by about 9 to 10% in two experiments, and although excessive rainfall in July and August of 1939 increased development in central Gaspé populations by 4 to 5%, the bulk of the evidence from studies in central Gaspé and at Parke Reserve shows that the overwintered populations typically fail to respond in any perceptible degree to moisture addition after mid-June. The effect of unseasonably dry conditions in the spring is therefore to reduce still further the characteristically low development in the overwintered populations in a one-generation area.

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